# Histological Evaluation of Diode Laser Pulpotomy in Dogs

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**Purpose:** The purpose of this study was to evaluate the histological changes in dental pulp tissue after diode laser pulpotomy, with an attempt to standardise the duration of laser exposure time.

**Materials and Methods:** Forty-two premolars from six healthy 1-yr-old Coorg local breed dogs were selected. Pulpotomy was initiated after achieving adequate anaesthesia. Hemostasis was achieved by exposure to diode laser (810 nm) at 2 W for 1 s in group II, 3 s in group III, 5 s in group IV, while group I was not treated and used as negative control. The teeth were extracted at the end of 24 h and 7 days. Histological sections using hematoxylin and eosin-stained pulp tissues were made and evaluated by an optical microscope for tissue necrosis, extent of inflammation and extent of pulpal fibrosis, pulpal edema and vascular changes.

**Results:** A necrotic layer surrounded by both acute and chronic inflammatory cells was a common finding adjacent to pulp interface. Only one specimen in group IV showed signs of internal resorption in the dentinal wall. Intact odontoblasts were maintained in most of the specimens but were found disrupted in a few specimens in groups III and IV.

**Conclusion:** Most regressive changes were seen with 5-s laser application. One- and 3- s applications seemed to be ideal for diode laser pulpotomy as most of the specimens showed intact odontoblasts. Hence, diode laser therapy can be an acceptable alternative to the conventional pharmacotherapeutic methods of pulpotomy.

**Keywords:** pulpotomy, diode laser, exposure time, necrosis, fibrosis, lymphocytic infiltration.

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Pulpotomy entails the removal of infected coronal pulp followed by the placement of a suitable medication to preserve the health of remaining radicular pulp. Formocresol as a suitable medication in pulpotomy has remained a treatment of choice in primary teeth and is widely accepted.[1] But studies have demonstrated the potential for local and systemic distribution of formocresol with attendant potential toxic effects,[2,3]

such as mutagenicity,[2] carcinogenicity, immune sensitization,[4] and a definite relationship between formocresol pulpotomies and enamel structure changes in permanent successor teeth.[5,6] This led to the search for biocompatible substitutes, such as glutaraldehyde,[7] ferric sulfate,[8] electrosurgery,[9] mineral trioxide aggregate,[10] and laser irradiation.[11]

With the use of various types of lasers in pediatric dentistry, laser irradiation in vital pulp therapy has been proposed as an alternative to conventional pharmacotherapeutic techniques. Their use in pulpotomies was first reported by Shoji in 1985, who used carbon dioxide laser.[12] The other lasers that have demonstrated moderate sucess in pulpotomy include carbon dioxide laser,[13] Nd:YAG[14] and Er:YAG laser.[15] Recently, diode laser was used for pulpotomy in primary teeth, which showed clinical success rates comparable to formocresol.[16]

The choice of laser use on pulp tissue is dependent on tissue resistant temperature values (TRT) of the pulp. The values of TRT are tissue specific and directly related to the water content and its vascularity.[17] Diode laser suits the TRT values of pulp due to its high absorbance at 810 nm wavelength, which avoids excessive heating and charring of pulp. Furthermore, diode laser is a contact laser, ie, the laser-emitting tip is applied in immediate contact with soft tissues, and hence only the site of application (micrometer range) is affected, leaving the remaining tissue unaffected. This offers treatment benefits including hemostasis, sterilization, and faster pulpal wound healing by its stimulating effects on the dental pulp cells[18] without affecting the inflammatory function of monocytes, endothelial cells, or the adhesion of endothelial cells.[19]

A recent in-vivo study reported no significant differences between diode laser/mineral trioxide aggregate (MTA) pulpotomy and formocresol/zinc-oxide-eugenol (ZOE) pulpotomy, when assessed for clinical and radiographic success.[16] The investigators had lased the pulp tissue until hemostasis was achieved, which evidently meant that the pulse and duration of exposure were not standardized. This prompted the present animal study to evaluate the histological changes in dental pulp tissue after diode laser pulpotomy, with an attempt to standardise the duration of exposure.

## MATERIALS AND METHODS

The present in-vivo study was carried out in the Department of Pedodontics and Preventive Dentistry, Coorg Institute of Dental Sciences, in association with Sanjeevini Laser Center, Mysore, Department of Veterinary and Animal Husbandry, Government of Karnataka, Virajpet and the Department of Oral Pathology and Histology, Coorg Institute of Dental Sciences. The protocol was reviewed and approved by the ethics committee.

Six healthy 1-yr-old Coorg local breed dogs were selected for the study with the aid of a veterinary surgeon (Govenment Animal Husbandry, Virajpet).

### Preparation of Dogs

The dogs were dewormed with pyrantel pamoate 10 mg/kg body weight (Nemolid®) and vaccinated with the combined vaccine (Nobivac-Dhppi+5L) against the common life-threatening diseases, eg, canine distemper, canine hepatitis, pavo viral gastroenteritis, para influenza, leptospirosis, and rabies with Raksharab® (Indian immunologicals). A total of 42 premolars from six dogs were selected and divided equally into the following groups as:

- Group I: Control (6 premolars)
- Group II: 1 s laser exposure (12 premolars)
- Group III: 3 s laser exposure (12 premolars)
- Group IV: 5 s laser exposure (12 premolars)

### Laser Device and Laser Parameters

A pulsed contact mode diode laser (MeDioStar XT; Fig 1), emitting at 810 nm, with pulse duration 500 ms, pulse energy 2 W, pulse rate 0.5 Hz, was used.

### Pulpotomy Procedure

#### *1. Animal Preparation*

Animals were fed with solid food 12 h before the procedure and were kept nil per oral 6 h before the procedure.

For the sake of convenience and in order to prevent any contamination of the between various experimental groups, the six dogs were coded as follows:

- D 11: 1-s laser application and extraction after 24 h
- D 13: 3-s laser application and extraction after 24 h

- D 15: 5-s laser application and extraction after 24 h
- D 21: 1-s laser application and extraction after one week
- D 23: 3-s laser application and extraction after one week
- D 25: 5-s laser application and extraction after one week
- D1 and D 2 represent extraction of sample after 24 h and one week of the procedure and 1, 3, 5 represent duration of exposure.

***2. Anaesthetizing the animals***

The animals were pre-medicated with intravenous TrifluroPromazine (0.1–0.25 mg/kg body weight) (siquil®). Induction and maintenance of anesthesia was done using inravenous thiopentone sodium (10-20 mg/kg) (thiosol®) as 25% solution by a veterinary surgeon.

Once anesthesia was achieved, the cavity preparation was done on the selected teeth and the pulp chamber was exposed using a high-speed airotor. The coronal pulp was removed using a slow-speed round bur under continuous water spray and spoon excavator followed by copius irrigation with saline. Primary hemostasis was achieved by placing sterile saline-soaked cotton pellets on the radicular pulp stumps under light pressure for 5 min. Complete hemostasis was achieved by exposing root canal orifices to diode laser (MeDiostar laser of 810 nm) which was delivered by optical fiber tip (Figs 2 and 3).

The exposure time of diode laser was 1 s in group II, 3 s in group III, 5 s in group IV.

Then the access cavity was sealed with reinforced zinc oxide eugenol cement (IRM).

Postoperatively, the animals were kept in the animal house and maintained with a balanced diet under the supervision of a veterinary surgeon.

## Extraction of the Samples

After 24 h, three dogs coded D11, D13, D15 were pre-medicated with intravenous TrifluroPromazine (0.1-0.25 mg/kg body weight) (siquil®). Induction and maintenance of anesthesia was done using intravenous thiopentone sodium (10-20mg/kg body weight) (thiosol®) as 25% solution by a veterinary surgeon. Eight premolars from each dog were extracted, of which 2 constituted the control group and the other 6 teeth belonged to the experimental group. All specimens after extraction were fixed in 10% formalin and fixed samples were subjected to histological evaluation. Postoperatively, the dogs were cared for by a veterinary surgeon.

**Fig 1** Diode laser 810 nm (MeDioStar XT).

After one week, 3 dogs coded D21, D23, D25 were pre-medicated with intravenous TrifluroPromazine (0.1–0.25 mg/kg body weight) (siquil®). Induction and maintenance of anesthesia was done using intravenous thiopentone sodium (10-20 mg/kg body weight) (thiosol®) as 25% solution by a veterinary surgeon. Six treated premolars from each dog were extracted which belonged to the experimental group. All specimens after extraction were fixed in 10% formalin and fixed samples were subjected to histological evaluation. Postoperatively, dogs were under the care of a veterinary surgeon.

## Histological Procedure

The specimens were fixed in 10% formalin for two days, and subsequently decalcified using 10% nitric acid. The teeth were later processed, sectioned and stained with hematoxylin and eosin for histological assessment.

The histological reaction of various samples was examined under optical light microscopy for tissue necrosis and extent, inflammation and extent, pulpal fibrosis, pulpal edema, and vascular changes.

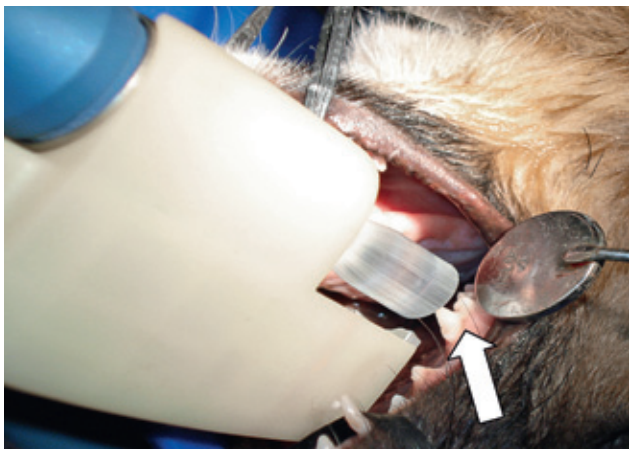

**Fig 2** Application of laser.

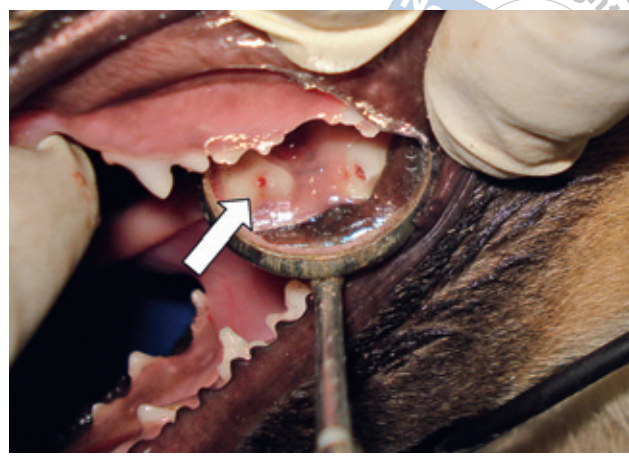

**Fig 3** Complete hemostasis.

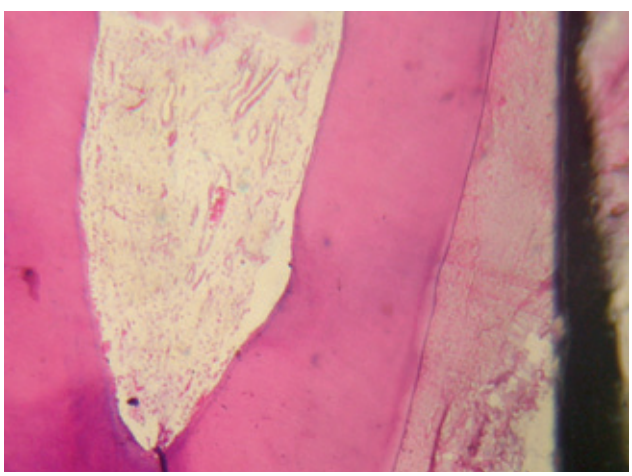

**Fig 4** Representative of group I showing fibrous connective tissue stroma that contained blood vessels.

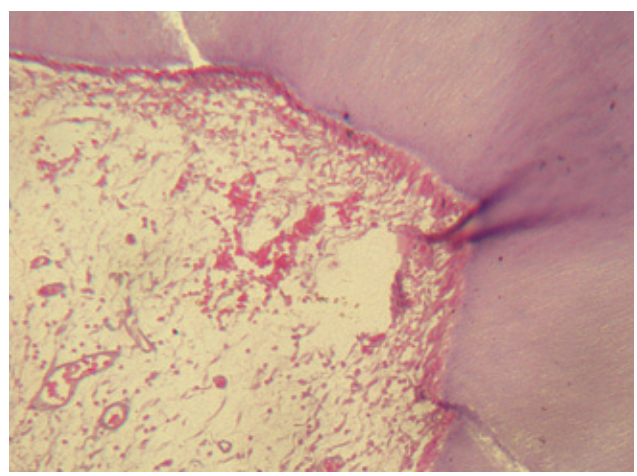

**Fig 5** Representative of group II (D11) showing mild hyperemic and intact blood vessels.

## RESULTS

Table I gives a summary of the histological reactions observed in the experimental groups.

### Histological Observations of Group I Samples (Fig 4)

All of the untreated teeth in the control group showed well-formed dentin, cementum, periodontal ligament, and pulpal chamber that included an odontoblastic layer rimming a delicate fibrous connective tissue stroma that contained blood vessels.

### Histological Observations of Group II Samples

#### *At the end of 24 hours (Fig 5)*

All the specimens in this group showed necrosis and inflammation which was mixed in nature, predominantly with chronic lymphocytes. The zone of necrosis and lymphocytic infiltration was limited to the point of application of the laser.

Adjacent areas below the zone of necrosis and lymphocytic infiltration showed hyperemia, edema, and decreasing inflammatory infiltrate. Intact odontoblasts were seen from the cervical third of the crown along the entire root.

**Table I Summary of the histological reactions observed in experimental groups**

| Group | Inflammation | | Dentinal debris | Pulp hyperemia | Zone of necrosis | | Intact odontoblasts | | Internal resorption |
|---|---|---|---|---|---|---|---|---|---|
| | Acute | Chronic | | | Crown | Root | Crown | Root | |
| D11 | | mixed predominately chronic lymphocyte | present mild | present | point of application | | cervical third of crown to entire root | | No |
| D21 | | chronic inflammation | present mild | present | point of application | | cervical third of crown to entire root | | No |
| D13 | mixed predominately acute inflammmatory neutrophils | | present mild | present extending to root tips | point of application | | | entire root | No |
| D23 | | mixed inflammation | present moderate | present extending to root tips | point of application | | | entire root | No |
| D15 | | mixed inflammation | present moderate | present | point of application | | | apical half of root | No |
| D25 | | chronic inflammation | present moderate | fibrosis in pulp | | extending deeper into roots | | apical half of root | resorption of dentinal wall |

***At the end of 7 days (Fig 6)***

All the specimens in this group showed necrosis and inflammation, which was chronic and varied from mild to moderate. The zone of necrosis and lymphocytic infiltration was limited to the point of application of the laser.

Adjacent areas below the zone of necrosis and lymphocytic infiltration showed mild hyperemia, edema and decreasing inflammatory infiltrate. Intact odontoblasts were seen from the cervical third of the crown along the entire root.

## Histological Observations of Group III Samples

***At the end of 24 hours (Figs 7 and 8)***

All the specimens in this group showed necrosis and inflammation, which was mixed in nature, predominantly with acute inflammmatory neutrophils. The zone of necrosis was limited to the point of application, and lymphocytic infiltration was seen extending to the middle third of the pulp.

All the samples had intact odontoblasts seen throughout the entire root, except in one sample which had a degenerating odontoblastic layer in the area adjacent to the point of laser application. Adja-

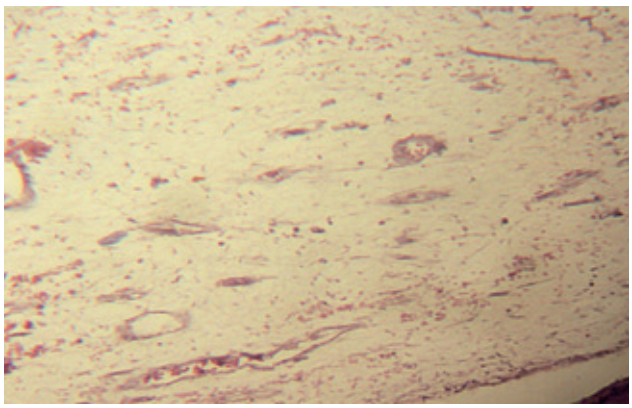

**Fig 6** Representative of group II (D21) showing decreased inflammation and edema.

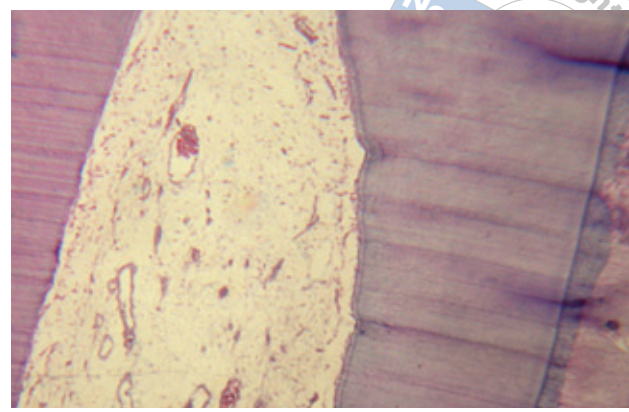

**Fig 7** Representative of group III (D1 3) showing edematous pulp tissue in rest of root canal.

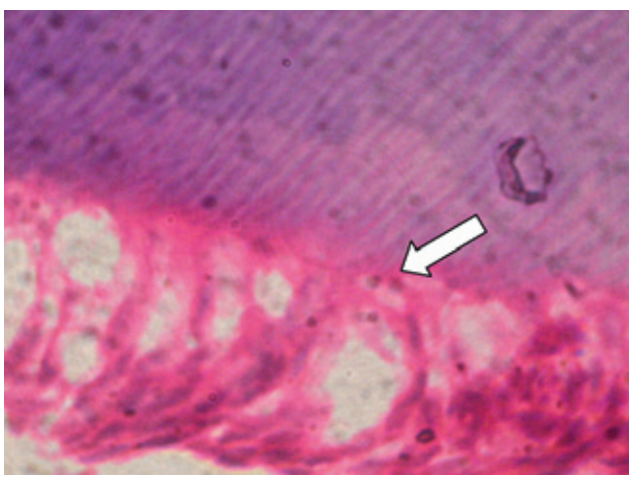

**Fig 8** Representative of group III (D1 3) showing degenerating odontoblastic layer.

cent areas below the zone of necrosis and lymphocytic infiltration showed hyperemia edema and decreasing inflammatory infiltrate extending up to the apical area.

### *At the end of 7 days (Figs 9 and 10)*

All the specimens in this group showed necrosis and inflammation, which was mixed in nature. The zone of necrosis with dentin debris and mild to moderate inflammatory infiltate was seen extending up to the middle third of the pulp.

All the samples had intact odontoblasts seen throughout the entire root length. Adjacent areas below the zone of necrosis and lymphocytic infiltration showed hyperemia, edema and decreasing inflammatory infiltrate extending up to the apical area.

## Histological Observations of Group IV Samples

### *At the end of 24 hours (Figs 11 and 12)*

All the specimens in this group showed the greatest extent of necrosis and inflammation, which was mixed in nature when compared to other groups. The zone of necrosis was limited to the point of application and lymphocytic infiltration was seen extending up to the middle third of the pulp.

All the samples had intact odontoblasts in the apical half of the root, except in one sample which lacked an odontoblastic layer with space between the dentin and pulp, showing extravasated red blood cells (Fig 13). Adjacent areas below the zone of necrosis and lymphocytic infiltration showed hyperemia edema and

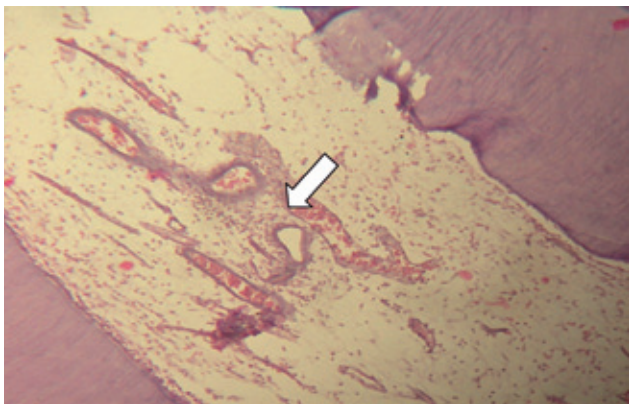

**Fig 9** Representative of group III (D2 3) showing middle 1/3 of hyperemic pulp.

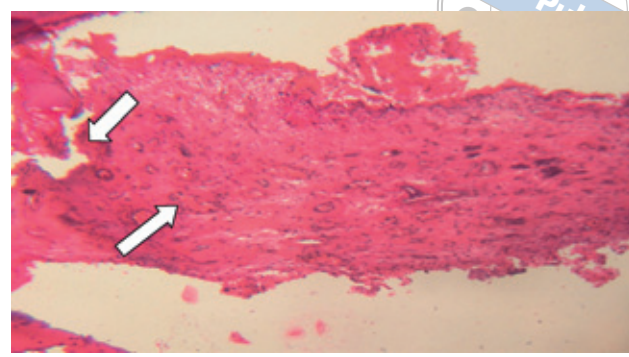

**Fig 10** Representative of group III (D2 3) showing pulp necrosis, inflammation and rest of pulp tissue shows enlarged blood vessels.

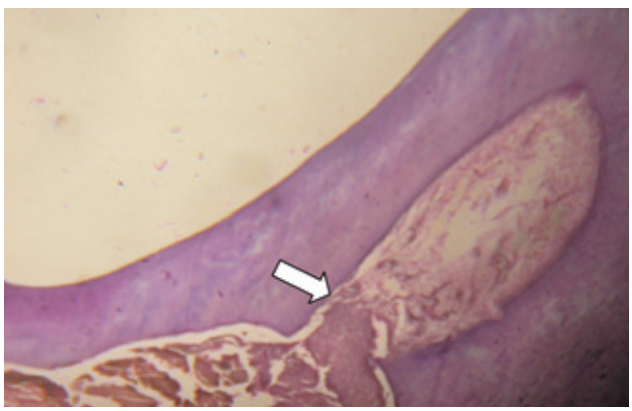

**Fig 11** Representative of group IV (D1 5) showing junction of necrotic area with normal pulp tissue in root canal.

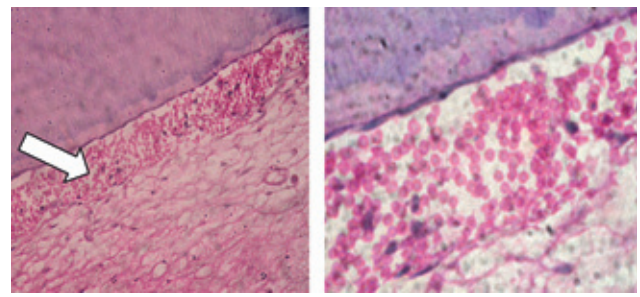

**Fig 12** Representative of group IV (D1 5) showing absence of odontoblastic layer.

decreasing inflammatory infiltrate extending up to the apical area.

## Histological Observation of Samples Extracted at the End of 7 Days (Figs 13 and 14)

All the specimens in this group showed the greatest extent of necrosis and inflammation, which was chronic in nature when compared to the other groups. The zone of necrosis was limited to the point of application and inflammation was seen extending throughout the entire pulp.

The four samples had intact odontoblasts seen in the apical half of the root. Two specimens showed evidence of pulpal fibrosis. Adjacent areas below the zone of necrosis and lymphocytic infiltration showed hyperemic edema and decreasing inflammatory infiltrate extending deeper into the roots. One specimen in this group showed signs of internal resorption in the dentinal wall (Fig 14).

A necrotic layer surrounded by both acute and chronic inflammatory cells was a common finding adjacent to the pulp interface. Only one specimen in group IV showed signs of internal resorption in the dentinal wall. Intact odontoblasts were maintained in most of the specimens and disrupted in a few other specimens in groups III and IV.

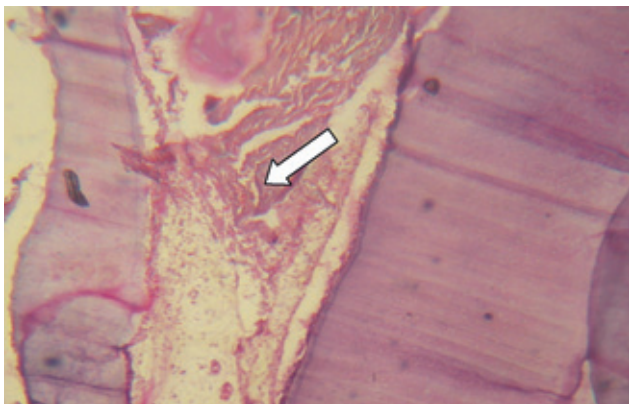

**Fig 13** Representative of group IV (D2 5) showing lymphocytic infiltration and hyperemic edema.

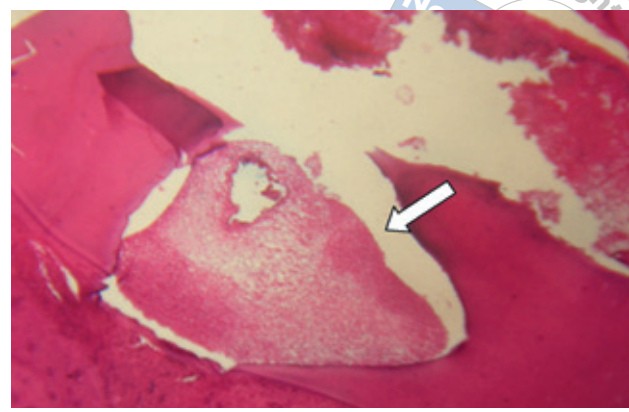

**Fig 14** Representative of group IV (D2 5) showing areas of resorption and adjacent side of pulp show fibrosis.

## DISCUSSION

Pulpotomy is advocated in primary and young permanent teeth with pulp exposure either due to caries or trauma. It ensures the removal of the infected coronal pulp and maintains the vitality of remaining radicular pulp. Based on the medications used, pulpotomy is classified by Ranly as: devitalization (formocresol, glutaraldehyde, electrocoagulation), preservation (ferric sulphate, calcium hydroxide, mineral trioxide aggregate, lasers) and regeneration (indirect pulp therapy, bone morphogenic proteins, collagen).[20]

An ideal pulpotomy medication should preserve the vitality and function of radicular pulp. Histologically, it should show an intact odontoblast-lined dentin chamber. This helps the tooth to enter into the exfoliative process at the appropriate time.[21] From the review of various medications used for pulpotomy, it was observed that formocresol, ferric sulphate and MTA were the medications of choice.[22]

Formocresol pulpotomy is successful clinically. Its histological reactions are tissue fixation, coagulation necrosis and vital pulp. Beaver[23] reported that formocresol initially fixes the tissue on which it has been topically applied. As it diffuses apically, areas of coagulation necrosis, dilated blood vessels and inflammation appear. Block[24] showed that coagulation necrosis was followed by liquefaction necrosis of the pulp. This liquefaction was caused by the release of powerful hydrolytic enzymes from the dying neutrophilic leukocytes which ultimately led to periapical abscess with resorption of root surface.

MTA used as a pulpotomy agent revealed signs of internal resorption, which may have resulted from overstimulation of the primary pulp by its highly alkaline nature. This alkaline-induced overstimulation could have caused metaplasia within the pulp tissue, leading to the formation of odontoclasts and chronic pulpal inflammation.[25] The incidence of inflammatory root resorption and pulp necrosis following ferric sulfate pulpotomy has discouraged most clinicians from investigating this technique further.[21] Despite mutagenic and toxic effects, the devitalization approach of formocresol pulpotomy is still considered as the treatment of choice in primary teeth.[26]

The search for conservative, reparative and biological approaches to pediatric pulp therapy over the devitalization approach of formocresol pulpotomy led to the use of lasers. Laser has advantages when used as a pulpotomy method, such as control of hemorrhage, sterilization, preserving the vitality of the dental pulp, and faster pulpal wound healing by stimulation effects on the dental pulp cells, without affecting either the inflammatory function of monocytes and endothelial cells or the adhesion of endothelial cells.

The lasers commonly used for pulpotomy procedure are carbon dioxide,[13] Nd:YAG,[14] Er:YAG,[15] and Er,Cr:YSGG.[27] Clinical, radiographic, and histopathological evaluation of the effects of all these lasers demonstrated moderate success in comparison with formocresol.

Saltzman[16] and Matsui[18] demonstrated beneficial effects of diode laser on dental pulp tissue. Further, Matsui[18] showed activation of cells that promote

mineralization of human dental pulp cells. In the diode laser, energy is released as photons, while in most other semiconductor materials, energy is released as heat, which may damage the remaining radicular pulp tissue. This makes diode laser, known for its suitability for soft tissue incision and ablation,[17,28,29] also useful as a pulpotomy agent.

Shoji[12] reported that thermal injury to the pulp depends on the length of laser exposure to the pulp rather than the output power of the device to prevent irreversible pulpal damage. This necessiated standardization of the exposure time with lasers in pulpotomy. Thus, in our study, the selected diode laser exposure times were 1, 3 and 5 s.

Jengfen Liu14 found clinical success of pulpotomy procedure with Nd:YAG laser at 2 W. Shoji[12] concluded that bleeding from the pulp was prevented when $CO_2$ laser at ≥3 Joules was applied. Saltzman[16] used diode laser with 3 W until hemostasis was achieved and reported less radiographic success compared to formocresol pulpotomy. Thus, in our study, the lasing energy of diode laser selected was 2 W.

The use of formocresol in dog pulp tissue showed necrosis of the coronal portion of pulpal tissue next to a thin superficial fixation layer which indicated poor and inadequate fixation.[30] In our study, necrosis and inflammation, which were mixed in nature, were limited to the point of application (within the coronal third of pulp), with the remainig radicular pulp vital with 1- and 3-s exposure after 24 h and one week.

The periapical abscess with resorption of root surface was evident in dog teeth after formocresol pulpotomy indicating complete loss of vitality with fibrous granulation tissue in the apical third of the root canal.[30] Toomarian[27] found an abscess below the necrotic layer with intact odontoblasts in a few samples. In our study, intact odontoblasts were observed in all groups except a few samples of 3-and 5-s exposure which showed disrupted odontoblasts. Periapical abcesses were not seen in any of the groups. This could be attributed to the minimal exposure time and penetration of diode laser into the pulp tissue. The depth of penetration reported with the diode laser was 100 to 300 μm.[16]

A dentin bridge was not noticed in any of our samples. However, the formation of teritiary dentin as a response to GaAlAs laser irradiation was noticed by influencing the secretory activity of odontoblasts[31] or activating the cell signaling molecules, such as Smads (a class of proteins that modulate the activity of transforming growth factor beta ligands) that promote mineralization of human dental pulp cells.[18] Jukic[32] reported that Nd:YAG laser irradiation caused necrosis, carbonization, inflammatory infiltration, edema, and hemorrhage in the pulpal tissue of dogs. In our study with diode laser, necrosis was limited to the point of application in all the groups and no carbonization was seen, probably due to the use of pulsed laser with limited lasing duration of 1, 3 and 5 s.

In our study, predominately mixed chronic inflammatory lymphocyte was seen in all groups except in 3-s exposure after one day, where predominately mixed acute inflammmatory neutrophils were seen, but the extent of inflammation in 1-s exposure was limited to the point of application, while in 3-s exposure, the extent of inflammation was limited to the middle third of the pulp. In 5-s exposure, the inflammation extended into whole of pulp. This is in keeping with Shoji,[12] who states that thermal injury to the pulp depends on the length of pulpal exposure to the laser rather than the output power of the device.

After 7 days, only two specimens with 5-s exposure showed evidence of pulpal fibrosis indicating reversible damage. In contrast, Toomarian[27] noticed after 7 days in some cases that abscess formed below the necrotic layer, indicating irreversible damage.

One specimen with 5-s exposure after one week showed resorption of dentinal wall, which agreed with Toomarian[27] about Er,Cr:YSGG laser irradiation demonstrating internal resorbtion with odontoclasts. In our study, a minute amount of dentinal debris was found in all the groups.

## CONCLUSIONS

Within the parameters used in this study, diode laser pulpotomy showed favorable results. Diode laser exposure of the dental pulp for 1 and 3 seconds showed better integrity of the odontoblastic layer, less inflammation and reduced levels of tissue necrosis and resorption. Diode laser exposure of the dental pulp for 5 seconds showed the greatest amount of regressive changes. One- and 3-s applications of diode laser appear to be the ideal exposure times for diode laser pulpotomy.

In light of the present findings, it appears that diode laser therapy could be an acceptable alternative to conventional pharmacotherapeutic techniques for pulpotomy. However, we recommend future long-term clinical studies on a larger sample group with evaluation of radiographic and histologic success before human trials begin.

## ACKNOWLEDGMENTS

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