

## HISTOPATHOLOGICAL PULP RESPONSE TO PLATELET-RICH PLASMA PULPOTOMY IN PRIMARY TEETH

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### **ABSTRACT**

**Background:** To overcome the disadvantages of various synthetic-based biomaterials used in pulp therapy, the researchers are directed towards innovation of novel materials that are biologically compatible. Platelet-rich plasma (PRP) is considered one of the novel biologically-based autologous substitutes.

**Purpose:** To evaluate histopathologically the pulp response to the platelet-rich plasma as a pulpotomy medicament in primary teeth.

**Materials and methods:** A total of 30 healthy lower first primary molars and canines that indicated for serial extraction were selected for this experiment. PRP and formocresol pulpotomies were performed (12 teeth/each group), where the other 6 teeth were used as a control. Four teeth from each experimental group and one control were extracted at post-treatment intervals; 14 days, one and three months. The samples were prepared and the tissues' sections were stained with Hematoxylin & Eosin for histopathological evaluation. Using ordinary light microscope, all sections were histopathologically analyzed blindly in terms of soft tissues organization, degree of inflammatory reaction, hyperemic changes, pulp necrosis and dentin bridge formation.

**Statistical analysis:** All data were analyzed with the Mann-Whitney U-test and the probability level of significance was accepted at  $p < 0.05$ .

**Results:** The overall histopathological results of pulp response to PRP were statistically significant in terms of soft tissue organization, degrees of inflammation, hyperemia, and pulp necrosis than formocresol ( $p < 0.05$ ).

**Conclusion:** Histopathologically, it was concluded that PRP had induced significantly a successful healing biologic response than synthetic formocresol; serving as a potent autogenous pulpotomy medicament alternative to formocresol.

**KEYWORDS:** Platelet-rich Plasma, Histopathological response, Formocresol pulpotomy, Primary dentition.

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## INTRODUCTION

The vital pulpotomy is considered as an emergency intervention for transient relief of acute symptoms and maintenance of the tooth and arch integrity<sup>(1)</sup>. It is aimed to treat the reversible inflamed coronal pulp and maintaining the radicular pulp healthy<sup>(2)</sup>, therefore, facilitating the primary dentition to normal exfoliation.

Formocresol (FC) is the gold standard pulpotomy technique in pedodontics since 1904<sup>(3,4)</sup>. However, formocresol has been categorized as a carcinogen<sup>(4)</sup>, in addition to the mutagenic, immunogenic and toxicity potential of formaldehyde<sup>(5)</sup>. Other several investigated techniques including pharmacotherapeutic and non-pharmacotherapeutic approaches showed positive effectiveness outcomes<sup>(6)</sup>.

In spite of the technological revolution in the field of synthetic-based dental restoratives, the available data still reported their mild to moderate cytotoxicity on pulpotomized teeth. Therefore, it is essential to develop novel biocompatible and biologically based therapeutics that maintain pulp vitality<sup>(7)</sup>, neutralize the side effects of previously used synthetic based biomaterials, increase tooth integrity by energizing of body's natural healing, and form biological tissue<sup>(8)</sup>.

In dental medicine, a modern innovation has been prepared using components of self-patient blood; platelet-rich plasma (PRP)<sup>(5)</sup>. It has been widely used in medicine as a catalyst for the wound and soft & hard tissues healing since 1970s<sup>(9,10)</sup>. This therapeutic concentrate of the 1<sup>st</sup> generation is considered a promising autologous, an innovative tool, and a biologically active derivative. It combines the power of novel chemical, physical and biological science for actual problems solving in clinical pedodontics<sup>(5)</sup>.

The PRP properties are based on the release and production of differentiation factors, bioactive molecules, and multiple growth factors (GFs) onto

platelet activation. Such factors are extremely critical in both stimulation and regulation of wound healing process<sup>(11)</sup>.

Also, they have a significant effect in promoting and organizing cellular processes such as metabolism, differentiation, proliferation, chemotaxis, vasculogenesis, and mitogenesis<sup>(12)</sup>. The growth factors interact with each other, thus producing a cascade of various signal proteins with multiple pathways, and finally leading to protein production with activation of gene expression<sup>(13)</sup>.

Because PRP can be obtained in a short time from the patient's self-blood, it seems to be an economical, cost-effective, safe, and aseptic method<sup>(10)</sup>. The use of this concentrate in vital pulp therapy has not been encountered, though it is used in many dental branches such as oral and maxillofacial surgery, and periodontology<sup>(14)</sup>. Until now, there are very few outcomes concerning the effects of using these endogenous growth factors on pulp therapy, and they are still often questionable<sup>(15)</sup>. So, the objective of this article was to evaluate histopathologically the pulp response to the platelet-rich plasma as a pulpotomy medicament in primary teeth.

## MATERIAL AND METHODS

For histopathological examinations, a total of thirty healthy lower first primary molars and canines that formerly planned for serial extraction were selected from children who attended the outpatient Clinic of Pedodontics, Faculty of Dentistry, Tanta University. A split-mouth design was used where one side was randomly selected for one group intervention and the contralateral side for the other. Platelet-rich plasma and formocresol pulpotomies were performed (twelve teeth for each group), and the other six teeth were used as a control. To maintain clinical standardization, all clinical interventions were achieved by a single investigator. Four teeth from each experimental group and one control were extracted at post-treatment intervals;

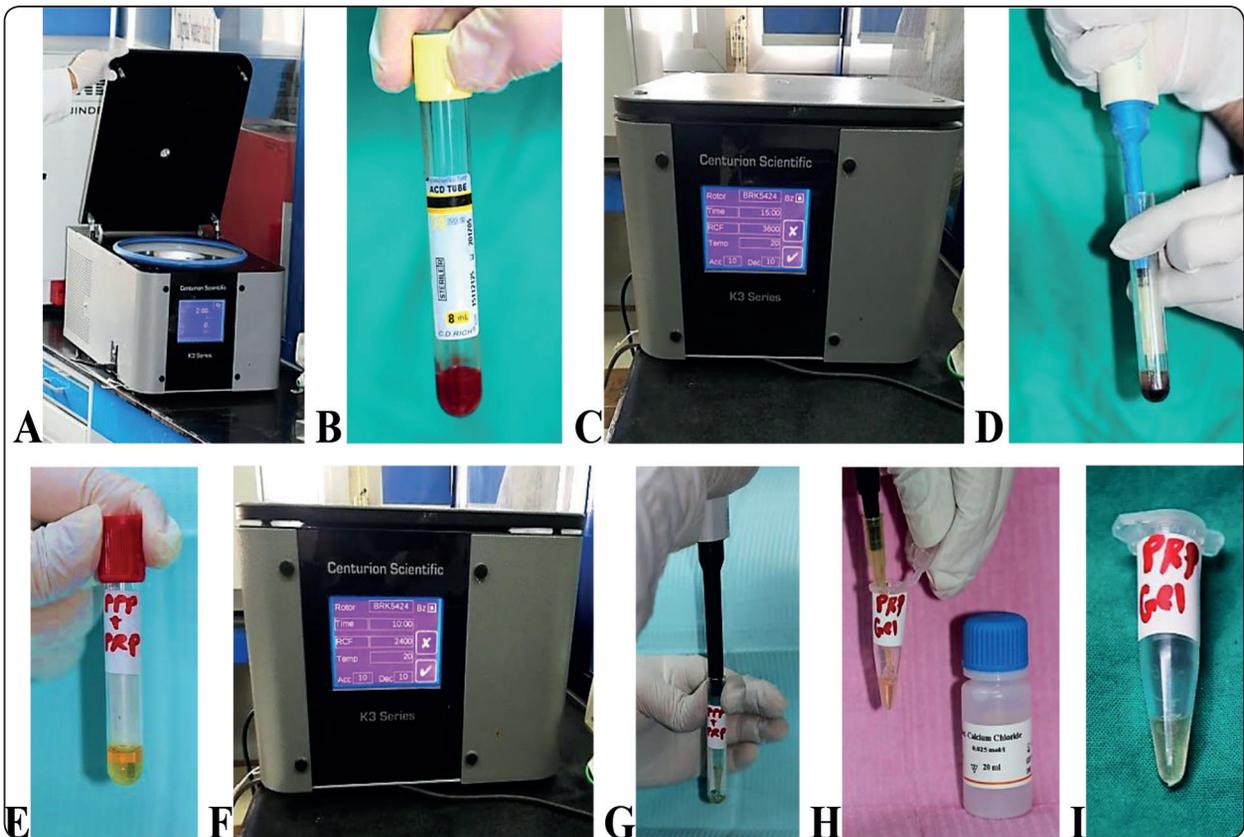
two weeks, one and three months. Approval of this research was documented from the Research Ethical Committee, Faculty of Dentistry, Tanta University. The clinical procedure was explained to the parents and informed consent was a signed and ascent from children above 8 years was taken.

### The procedure of PRP gel preparations

Thirty minutes prior to the clinical procedure, a sample of peripheral venous blood (1ml) was drawn from the patient and directly transferred into a sterile evacuated polystyrene tube containing Acetic acid Citrate Dextrose (ACD TUBE, C.D. RICH) as an anticoagulant and for the preservation of high platelets concentrate. The tube was inverted about 5 times before centrifugation.

The citrated whole blood sample was exposed to the first centrifuge at 2,400 rpm for 10 min using Centurion Scientific Benchtop centrifuge (UK) (Fig 1A). At this step, the sample was separated into three layers: the top, mostly PPP with few leukocytes; the middle, thin PRP with rich leukocytes; the bottom, mostly red blood cells (RBCs) (Figs 1B-1D). Using a sterile microbipite, both PPP and PRP layers were collected and transferred into a 5ml sterile glass tube without anticoagulant that undergoes a second centrifuge at 3,600 rpm for 15 min. The concentrated PPP at the upper 2/3<sup>rd</sup> of the tube was discarded leaving the concentrated homogenized platelet pellet at the bottom <sup>(16-18)</sup> (Figs 1E-1G).

Before the clinical intervention, PRP was activated for 15 min in 1.5 ml Eppendorf tube with



Figs. (1A-I) Steps for PRP Gel preparation: (A) Centurion Scientific Centrifuge. (B) A sterile ACD tube contained 1ml of the peripheral venous blood. (C) The first centrifuge program. (D) Aspiration of PPP and PRP layers using a sterile microbipite. (E) A sterile glass tube contained the collected PPP and PRP. (F) The second centrifuge program. (G) Separation of concentrated pellets from the bottom. (H) Mixing of PRP with  $\text{CaCl}_2$ . (I) PRP gel.

50µl of 10% calcium chloride (CaCl<sub>2</sub>, Biomed, Egypt) per ml of PRP)19-22) (Figs 1H&I).

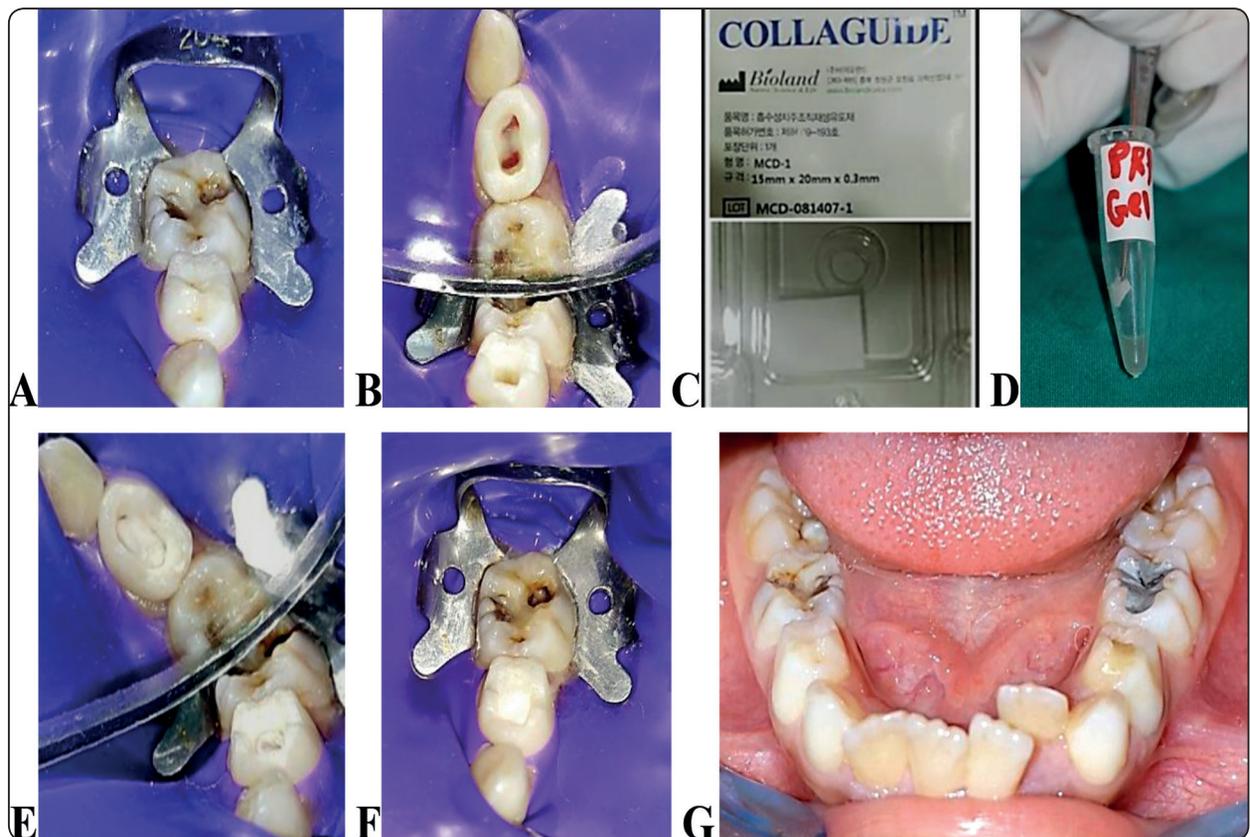
**Pulpotomy procedure**

After achieving profound local anaesthesia and rubber dam isolation, conventional pulpotomy technique was performed. A #2 diamond round bur mounted at high speed with air-water spray was used for preparing the access cavity, pulp chamber removal, and overhanging dentin elimination. Sharp a sterile spoon excavator was used to amputate the coronal pulp tissues followed by irrigation with normal saline. Initial pulpal haemostasis was obtained using a sterile moistened cotton pellet gently pressed against the amputated pulp stumps for 5 min. In PRP group, after achieving haemostasis

, PRP gel was carried with a previously trimmed highly absorbable sterile dental collagen membrane<sup>(23)</sup> (CollaGuide/Korea) that was placed directly on the pulp stumps and adjusted to the entire pulp chamber floor.

In FC group, a sterile cotton pellet was moistened with Buckley’s formula; the excess was removed by damping the pellet in gauze, and then placed on the stumps of amputated pulp for 5 min<sup>(24)</sup>.

The prepared cavities of all treated teeth were sealed with intermediate restoration (IRM, Caulk Dentsply, Milford, DE, USA) and subsequently covered with glass-ionomer Ketac Molar filling (3M ESPE, USA) according to manufacturer recommendation (Figs 2A-G).



Figs. (2A-G) Clinical steps of PRP pulpotomy on the lower left 1<sup>st</sup> primary molar that indicated for serial extraction: (A) Preoperative photograph. (B) Pulp chamber removal. (C&D) Absorbable sterile collagen membrane was used to carry out PRP GEL. (E) PRP with the collagen membrane on pulp stumps. (F&G) Sealing the cavity with IRM and finally restored with glass-ionomer filling.

### Histopathological evaluation

After the pulpotomized and negative control primary teeth were extracted, they were fixed in 10% formalin buffered solution for 10 days and then decalcified in 10% Ethylene diaminetetraacetic acid (EDTA) for 8 weeks after sealing the apical foramina with sticky wax. The decalcified specimens were dehydrated through gradually ascending concentrations of ethanol, and embedded in paraffin blocks. Serial longitudinal sections at 4- $\mu$ m thickness using a microtome was obtained, stained with hematoxylin and eosin (H&E), and then they were consequently examined histologically under ordinary light microscope (Eclipse 80i; Nikon, Tokyo, Japan). All sections were histopathologically analyzed blindly according to the modified criteria of Hasheminia *et al.*<sup>(25)</sup> and Songsiripraduboon *et al.*<sup>(26)</sup> for soft tissue organization, the degree of pulpal inflammation, hyperemic changes, tissues necrosis, and dentinal bridge formation.

### Histopathological criteria:

#### According to soft tissue organization:

- 1- Normal or almost normal organization under the exposure area, tissue-material interface or under the formed dentinal bridge. The presence of newly formed collagen fibers and well organized odontoblast-like cells.
- 2- Partial loss of normal organization under the exposure area, tissue-material interface, or dentinal bridge. Some collagen fibers and few cells appeared away from the exposure area.
- 3- Complete loss of normal organization and some free spaces were present.

#### According to the degree of pulpal inflammation:

- 1- Absence or little of inflammation: 1-3 inflammatory cells under exposure area or the dentin bridge.
- 2- Mild inflammation: 4-10 inflammatory cells under the exposure area or dentin bridge.

- 3- Moderate inflammation: 11-50 inflammatory cells under the exposure area or dentin bridge.
- 4- Severe inflammation: > 50 inflammatory cells or the presence of microabscesses under the exposure area or the formed dentinal bridge.

#### According to dentinal bridge formation:

- 0- Absence of coverage.
- 1- Partial coverage of the exposure area.
- 2- Complete coverage of the exposure area.

#### According to hyperemic changes:

- 0- Absence of congested blood vessels.
- 1- Mild: 1-3 congested blood vessels.
- 2- Moderate: 3-5 congested blood vessels.
- 3- Severe: > 5 congested blood vessels.

#### According to pulp tissues necrosis:

- 0- Absent.
- 1- Present.

#### Statistical Analyses:

All data were statistically analyzed using the SPSS program, version 20.0 (IBM, Illinois, Chicago, IL, USA). Based on the polymerized light microscopic The scores of histopathological features were analyzed by Mann-Whitney U-test. The probability level of significance was accepted at  $p < 0.05$ .

### RESULTS

A total of 24 pulpotomized primary 1<sup>st</sup> molars and canines with 6 untreated controlled teeth were evaluated histopathologically at post-treatment interval of 14 days, one and three months.

Based on the ordinary light microscopic examination, in control group, the odontoblastic cell layers demonstrated regular arrangement, and the pulp tissues displayed normal organization without inflammations, with the absence of hyperemic changes (capillaries dilatation) (Fig 3).

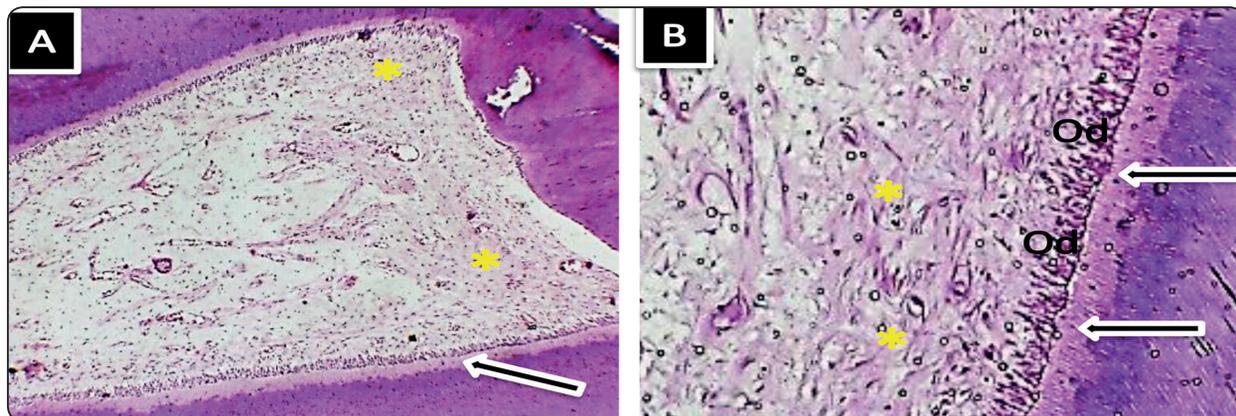


Fig. (3) Photomicrographs of control group showing normal pulp tissue organization, a layer of predentin (arrow) with high vascularity of pulp core (stars\*) and regular arrangement of odontoblastic cell layer (Od) [A]. (H&E X 100). High magnification showing dentin layer, predentin layer lined by well-organized odontoblastic cell layer (arrows)[B].

The overall histopathological results of pulpal response to PRP and FC pulpotomies are summarized in table 1. The outcome of PRP group recorded significantly: better soft tissues organization: cellular inflammatory response and: hyperemic changes than FC group ( $p < 0.05$ ) (table 1).

Most PRP stained sections 9 of 12 (75%) displayed normal soft tissues organization without pulpal inflammation and absence of hyperemic changes, while 5 (41.7%) sections exhibited an early stage of osteodentin formation. In FC group, exaggerated histopathological changes were observed; loss of soft tissues organization, moderate

pulpal inflammation and necrosis in 8 (66.7%) sections, with no evidence of any formed dentin bridge during all time-intervals.

At 14-day of recall-interval, in PRP group, partial loss of normal organization with moderate inflammation, mild to moderate hyperemic changes and irregular odontoblastic-like cells with mild fibrosis were revealed (Fig 4). In FC group, there was loss of tissue organization, moderate to severe pulpal inflammation & hyperemic changes and degenerated odontoblastic cell layers without any formed dentin bridge (Fig 5).

Table (1) The overall histopathological results of pulpal response of the experimental groups.

Groups	Soft tissue organization			Degree of inflammation				Hyperemic changes				Tissues necrosis		Dentin bridge formation		
	1	2	3	1	2	3	4	0	1	2	3	0	1	0	1	2
PRP no=12	9	3	-	9	1	2	-	9	1	2	-	12	-	7	5	-
FC no=12	-	4	8	-	-	8	4	-	-	7	5	4	8	12	-	-
X <sup>2</sup>	10.34			13.81				14.65				12.44		6.743		
P value	0.04*			0.02*				0.01*				0.03*		0.57		

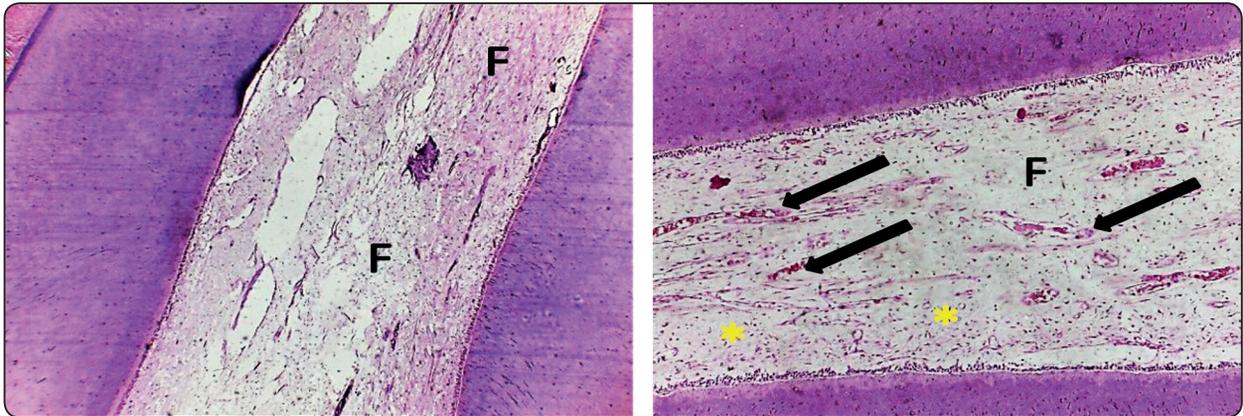


Fig. (4) Photomicrographs of PRP group after 14 days post-treatment interval showing partial loss of tissue organization, increased the inflammatory cells infiltrate (stars\*), moderate hyperemia (arrows) and increase the fibrosis (F). (H&E X100).

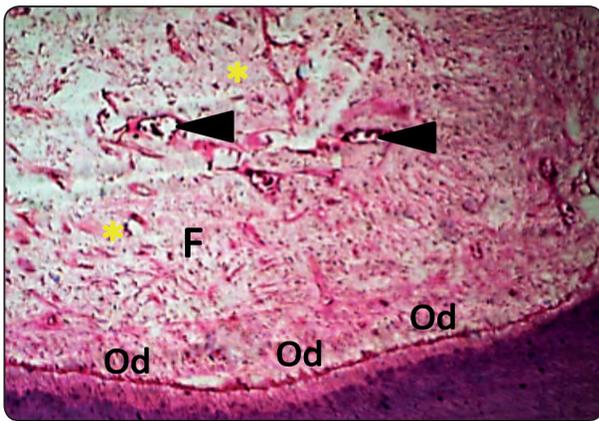


Fig. (5) Photomicrograph of the FC group after 14 days showing less organized odontoblastic layer (Od), increases the inflammatory cells infiltrate (stars\*) and increases of pulp hyperemia (arrows heads) with high fibrosis (F). (H&E X400).

After one-month interval, in PRP group, one section revealed partial loss of normal organization, mild inflammatory cellular infiltrate, hyperemic changes denoted by capillary dilatation, and decreased areas of fibrosis with partial coverage of exposure site (osteodentin) (Fig 6). However, in FC group, the sections revealed partial to complete tissue loss of architecture, moderate to severe inflammatory cellular infiltrate, pulp hyperemic changes and increased areas of fibrosis with areas of tissue necrosis.

At the 3-month interval, the PRP group displayed normal pulp tissues architecture and organization, no evidence of inflammation or hyperemic changes

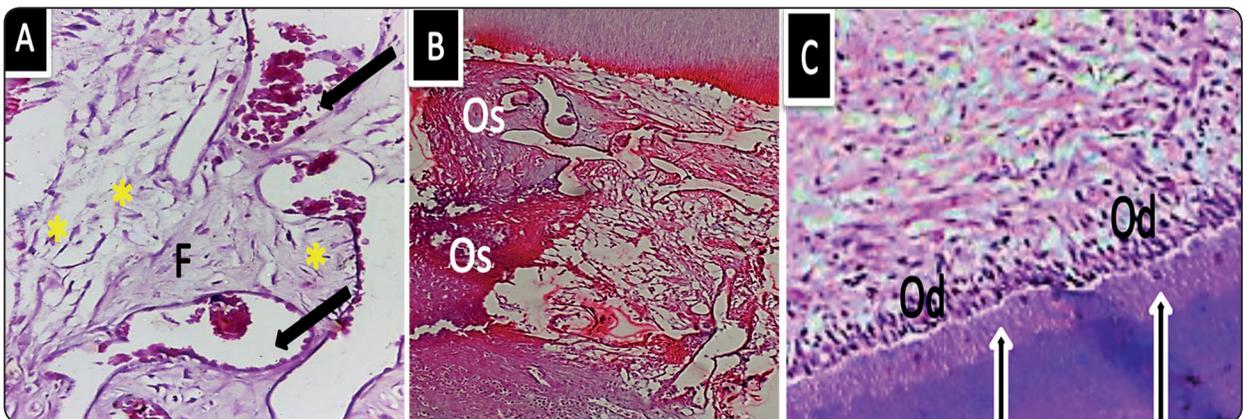


Fig. (6) Photomicrographs of PRP group, 1-month post-treatment interval showing mild inflammatory cells infiltrate (stars\*), mild hyperemia (arrows), increased fibrosis (F) [A], early stage of matrix formation (osteodentin) (Os) [B], and active odontoblast (Od) & wide band of predentin with active dentin formation (arrows) [C]. (H&E X400).

and intact odontoblastic-like cells (Fig 7). In FC group, complete disorganization of pulp tissues, severe inflammatory reaction, with hyperemic changes, areas of necrosis, and internal dentinal resorption were demonstrated (Fig 8).

Fig. (7) Photomicrograph of PRP section 3-month post-treatment interval showing well organized soft tissues with increased cellularity (stars \*), wide predentin band (arrows), odontoblastic layer (Od), and less of fibrosis. (H&E X400).

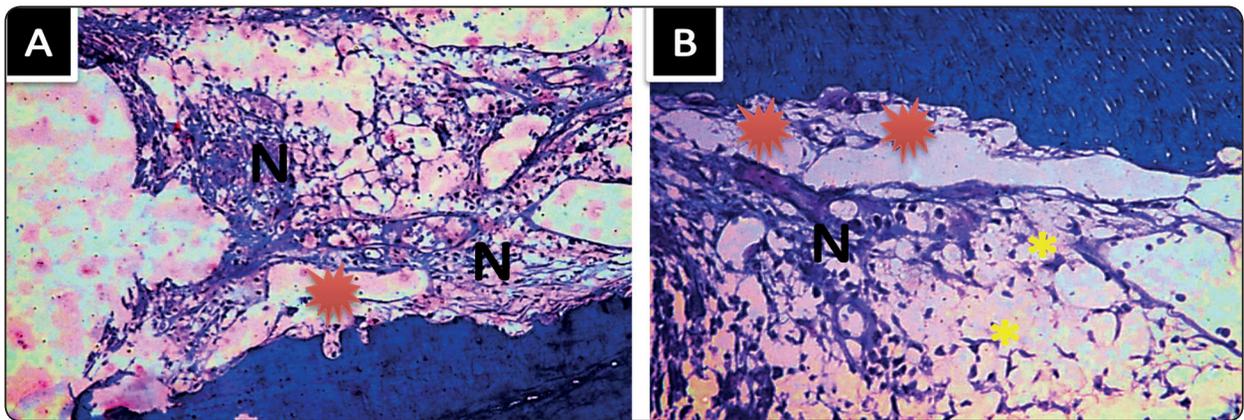
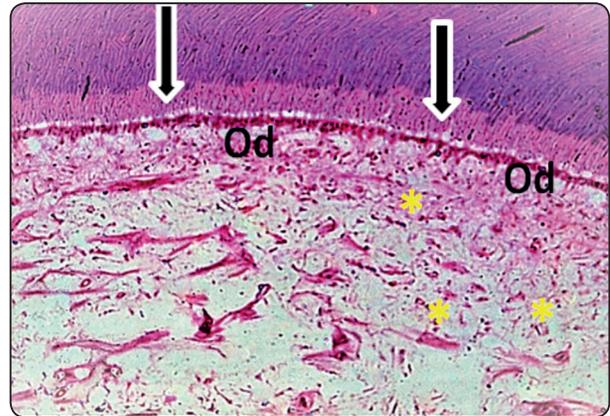


Fig. (8) Photomicrographs of FC section at 3-month post-treatment interval showing complete loss of organization, internal dentinal resorption ( \* ), and necrosis (N) [A]. High magnification showing inflammatory cell infiltrate (stars \*) and complete loss of odontoblastic integrity [B]. (H&E X200).

## DISCUSSION

It is essential to investigate any introduced novel dental biomaterial to clarify its percentage of success and safety to be approved for use by the clinician. The field of pulp therapy has undergone new innovations and several recent advances in materials and techniques <sup>(27)</sup>.

Nowadays, the attention and awareness of investigators are directed toward the regenerative medicine. Recently, PRP represented the attractive agent in this field, gained by sequestering and concentrating the platelets with its advantages of bioactivity and biocompatibility <sup>(28)</sup>. In addition, PRP is an easy, minimally invasive and low-cost procedure, producing high concentrations of autologous growth factors with cytokines into

damaged tissues in a physiological ratio <sup>(29,30)</sup>. All these advantages encourage the use of PRP as a pulpotomy medicament for primary teeth in this study.

The clinical and/or radiographic studies of any applied novel material are not sufficient for its overall evaluation. The histopathological studies are also highly recommended to determine the pulpal response to this material which may be difficult to be feasible in the human individuals, especially in pediatric age groups <sup>(31)</sup>. Until now, there is no histopathologic document about the pulpal response to PRP in primary teeth pulpotomy. So, the aim of this study was directed to evaluate the histopathological response of pulp to PRP compared to formocresol pulpotted primary teeth.

The acid-citrated Dextrose was used in the present study as an anticoagulant and for preserving the platelet viability via the prevention of platelet activation and degranulation<sup>(32)</sup>. Also, ACD tube was inverted about 5 times for proper mixing of the whole blood with the anticoagulant and avoiding the tiny fibrin clots formation that may lead to a falsely diminished platelet count<sup>(17)</sup>.

The two-spin centrifugal separation used for PRP preparation in the current study were; the initial low-speed spin for optimal separation of RBCs from the whole blood where the second high-speed spin for platelet concentration in small plasma volume at the bottom<sup>(17)</sup>.

In this study, the activation of the platelet by  $\text{CaCl}_2$  is a critical step. It affects the availability of various bioactive molecules, degranulates the platelets causing the gradual release of immediate stored GFs from  $\alpha$ -granules followed by a significant release of concentrated GFs including insulin-like, platelet-derived, transforming  $-\beta$ , and vascular endothelial growth factors with subsequently tissue healing process<sup>(9,33)</sup>. Moreover, it neutralizes the anticoagulant effect and converts fibrinogen into fibrin, results in platelet gel concentrate<sup>(34,35)</sup>.

The collagen membrane used in this study seems to be useful to survive PRP in the injured pulp tissues as much as possible and to accelerate and promote regeneration that may improve dramatically the clinical outcome<sup>(35,36)</sup>. Also, it was found that the active secretion of PRP's stored GFs (70%) starts within 10 min, approximating 100% within the first hour<sup>(32)</sup>. Furthermore, it acts as a barrier<sup>(37)</sup> preventing the unwanted restoratives to come into direct contact with vital pulp tissue protecting it from their undesirable forces during restorative techniques.

In the present study, formocresol was used as a control since it is still the popular and gold standard pulpotomy agent in primary dentition. This may be attributed to its simple use, easily available, better fixative efficacy, and bacteriostatic with highly clinical and radiographic outcomes<sup>(31,38)</sup>.

The finding of this study showed that PRP has a better soft tissue organization, and reduces cellular inflammation and hyperemic changes. This can be explained as the platelet counts exceed one million/ $\mu\text{L}$  in therapeutic PRP; 5 times more than that of the normal count<sup>(39)</sup>. In term, it forms a rapid merged fibrin clot or fibrin glue that adheres to the exposure site. In addition to its biodegradable and biocompatible characteristics, it prevents extensive tissue necrosis and fibrosis<sup>(5)</sup> through increasing the local cell division, inhibiting the excess inflammation by decreasing early macrophage proliferation, and containing synthesized and prepackaged GFs<sup>(40)</sup>; so, it plays a significant role in signaling the repair processes and formation in dentine-pulp complex. These autologous GFs provide a natural environmental condition for healing<sup>(38,41)</sup> and promote all process enhancing fast wound regeneration<sup>(5,42)</sup>.

These results also are in correspondence with Maden et al.<sup>(43)</sup> who found that there were statistically significant differences in cellular inflammation and hyperemic changes in the PRP group post-capping treatment in rats.

Although mild to moderate inflammations and hyperemic changes were obvious in PRP group, severe capillary dilatation or necrosis was not evident in any section. This is in accordance with the study of Maden et al.<sup>(43)</sup> who recorded the same findings, when they used PRP as a capping material.

In contrast, controlled studies on minipigs<sup>(44)</sup> and adult domestic pigs<sup>(45)</sup> demonstrated enhanced bone regeneration and healing with the application of PRP in sinus augmentation compared to the Beta-TCP without PRP<sup>(46)</sup>.

Growth factors signal many of the important events in tooth morphogenesis and differentiation<sup>(9)</sup>. They are considered the main stimulators and regulators of the stem cell niche as the keys links for growth and development<sup>(38,41,47)</sup>. In this study, post-PRP application; partial areas of osteodentin deposition over the exposure site were observed.

This may be due to the activity of odontoblast-like cells arising from the undifferentiated mesenchymal cells of the pulp, differentiating, organizing and depositing dentin-like structure<sup>(48)</sup>. This result is in compliance with the histological finding of Petrović et al<sup>(49)</sup> who demonstrated that PRP had a sufficient potential to promote a high-quality composition of dentin bridge with a maximal cell density.

In the current study, the use of formocresol displayed severe inflammation with capillary dilatation, areas of necrosis, marked fibrosis, and internal root resorption that indicate inadequate and poor outcomes<sup>(31,50)</sup>. These findings are in accordance with most previous studies<sup>(31,48,51)</sup> which reported severe pulpal inflammatory response to formocresol, reemphasizing the fact that tissue fixation by FC is incomplete<sup>(52)</sup>.

The marked increase of fibrous tissue, congestion of blood vessels and necrosis observed in FC group may be due to diffusion of the formaldehyde with its irritating effect through the radicular pulp. This is in agreement with Sant'Anna et al.<sup>(53)</sup> and Talaat et al.<sup>(48)</sup> who stated that formocresol promote inflammatory reaction with different mononuclear cells, dilatation of the vessels and coagulation necrosis that was detected, particularly in the lower two thirds of the root.

The internal dentinal resorption observed in FC group may be linked to the inflammation caused by application of formocresol which is considered a stimulating material with poor biocompatibility property<sup>(48,54)</sup>. The irritating effect of formaldehyde may lead to pulpal inflammation that stimulates and increases the numbers of odontoclasts and death of odontoblasts, initiating internal inflammatory dentinal resorption<sup>(55,56)</sup>.

In addition, complete loss of normal tissues organization and disruption of the odontoblastic layer were observed in FC group in this study. These findings are comparable to those obtained by Talaat et al.<sup>(48)</sup>, El- Meligy et al.<sup>(50)</sup> and Yorgancilar et al.<sup>(51)</sup> who found that the FC application leads

to lympho-mononuclear cells infiltration within connective tissues; thus, it can modulate immune and inflammatory responses in dental pulp. This finding may be explained the failure of dentin bridge formation during all time intervals.

Large sample size, long-time evaluation, and further investigations are highly recommended for analyzing the possibility of pulp reaction, understanding the inflammatory characteristics of PRP-induced pulp healing, confirming its biocompatibility and efficacy, and for the long-term prognosis of this novel autogenous treatment method

## CONCLUSION

According to the findings of this study, it seems that PRP promotes significantly better histological pulpal outcomes than formocresol Therefore; PRP has the potential for using as an alternative autogenous biocompatible medicament in human primary teeth pulpotomy.

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