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Effect of Tideglusib Drug as Capping Material by Inducing Stem Cell Differentiation in Rabbit Teeth

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ORIGINAL STUDY

Effect of Tideglusib Drug as a Capping Material on Inducing Stem Cell Differentiation in Rabbit Teeth

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Abstract

Purpose: This study was conducted to evaluate the effect of glycogen synthase kinase-3 inhibitors Tideglusib on stem cell stimulation for tooth repair. **Patients and methods:** Twenty-five adult male rabbits were divided into three main groups: control group I had 5 M without pulp exposure, mineral trioxide aggregate group II had 10 M with pulp exposure capped with mineral trioxide aggregate subdivided into group IIa capped for 4 weeks and group IIb capped for 6 weeks and Tideglusib group III had 10 M with pulp exposure capped by 50 nM Tideglusib dissolved and diluted in dimethyl sulfoxide carried by Kolspon sponge. Group III was subdivided into group IIIa, which was capped for 4 weeks and group IIIb which was capped for 6 weeks and sealed by resin-modified glass ionomer. Part of the samples was decalcified for 6 weeks in 19% EDTA for histological examination with Hematoxylin and Eosin stain and immunohistochemical stains. The other part was processed for transmission electron microscopic examination. **Results:** Histological results showed a distinct dentin bridge in Tideglusib group IIIb than the other groups, while the immunohistochemical stain with OctA-4 revealed high intensity at group IIIb. Lastly, ultrastructural results showed high activity of odontoblast cells and angiogenesis in Tideglusib group IIIb with preservation of vitality. **Conclusion:** Tideglusib is shown to promote the formation of reparative dentin through stem cell stimulation.

Keywords: Caries, Glycogen synthase kinase-3 antagonist, Stem cell, Tideglusib

1. Introduction

Dental caries is a prevalent chronic infectious disease resulting from tooth-adherent cariogenic bacteria that could metabolize sugars to produce acid, which over time demineralizes the tooth structure [1]. The prevalence of tooth decay continues to increase with growing urbanization and changes in living conditions. Dental caries remains highly prevalent in most developing countries [2].

WHO revealed that dental caries affected about 60−90% of children and adults in most developed nations, which is still an issue that received little attention [3]. According to census statistics from 2007, caries were found to be more prevalent among children from low-income families or members of racial or ethnic minorities, and they increased between 1988 and 1994 and 1999 and 2004 in children in the United States aged from 2 to 5 years [4,5].

Mineral trioxide aggregate (MTA) was a popular substance that caused reparative dentin (RD) formation with no pulpal inflammation. However, it was difficult to handle as it had poor adherence to the tooth structure and had a latent effect on tooth color, which prevented its practical application [6].

Dental pulp stem cells might be used in cell therapy to enhance pulp capping, causing dentin bridge formation beneath the exposed pulp [3]. Activating progenitor cells to speed up regeneration is the theoretical basis for dental tissue repair. Adult human teeth contained cells with MSC-like properties, indicating that these tissues served as significant adult stem cell reservoirs [6].

Teeth pose a significant challenge in regenerative medicine, due to their complex structure, which is crucial for both mastication and aesthetic reasons. Being the most natural and noninvasive source of

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stem cells, teeth are considered the most ethical tool for stem cell extraction [7–10].

Successful regenerative medicine relies on the application of signaling pathways for differentiation. It was proven that directed differentiation would resemble the signals that cells get as they were induced during different phases of development [11,12].

Ectodermal development could be initiated by molecular inhibitors of endogenous bone morphogenetic protein (BMP) and Wnt canonical signaling [13]. However, mesodermal differentiation has been initiated by transitory Wnt and low levels of transforming growth factor (TGF) family members. A greater activin A concentration could be necessary for endoderm formation [14].

There were many strategies for producing progenitor cells from each germ layer, including the following cells: chondrocytes, hepatocytes, renal cells, lung cells, motor neurons, and hepatocytes due to surgical accessibility, cryopreservation capability, higher generation of dentin tissues in comparison to non-dental stem cells, and their anti-inflammatory properties [15].

DPSCs are the most valuable dental source for tissue engineering [16]. These cells may be used in maxillofacial and orthopedic repairs, as well as reconstructions outside the mouth cavity. All tooth structures could be generated by DPSCs [17].

An excellent ex vivo model of a pulp-damaged tooth had shown that DPSC proliferated in the perivascular region before migrating to the lesion site [18]. These features were not seen with dentin injuries, showing that only pulp injuries with a compromised odontoblastic layer triggered the activation and migration of the DPSC [19].

The migration of DPSC to the wounded pulp site had been a complicated process that involved many interacted complex mechanisms. They must be induced first from their specialized position through signaling and growth factors [20].

There was not a clear marker that distinguished DPSCs from other MSCs. The expression rate of Chemotaxine (C-X-C motif) receptor 4 (CXCR4), granulocyte colony-stimulating factor receptor (GCSFR), and the cluster of differentiation 114 (CD114) antibody in fractionated DPSCs such CD31 and CD105+ cells was much higher [21].

Other stem cell marker expression included octamer binding transcription factor 4 (Oct3/4), Nanog, reduced expression protein 1(Rex1), growth differentiation factor 3 (GDF3), and signal transducer and activator of transcription protein (Stat3) occurred at similar levels in both DPSCs and CD105+ cells and the induced pluripotent stem cell (iPS) [22]. However, SRY box transcription factor 2 (Sox2) and CXCR4 mRNA expression in human DPSCs and pulp CD105+ cells are lower than that of iPS cells [22].

Dental lamina at the initiation stage and enamel knots from cap to bell stage (Dkk) family members interacted with Lrp5/Lrp6 co-receptors causing endocytosis of that complex, whereas secreted frizzled related protein (Sfrp) family members and Wif1 (Wnt inhibitory factor 1) inhibited the binding of Wnt ligands to Fzd receptors [23,24].

Wnt/β-catenin signaling [23] activated the expression of many negative Wnt regulators, including Axin2, Dkk1, and Sfrp1, which created a negative feedback loop to control the intensity and duration of Wnt activation [24].

While Akt/protein kinase B (PKB)-mediated phosphorylation of β-catenin was necessary to completely activate β-catenin signaling, protein kinase A (PKA) could phosphorylate β-catenin at the C-terminus to stabilize the protein [25].

The Wnt/β-catenin pathway was primarily active in dental epithelia during early tooth morphogenesis, including the dental lamina at the initiation stage and enamel knots from the cap to bell stage higher in dental pulp stem cells, suggesting that the Wnt/β-catenin pathway was inhibited in root resorption [23,24].

So, this rapid and easy natural tooth repair technique may result in a promising clinical tooth restoration method [11]. Therefore, glycogen synthase kinase (GSK-3) was a component of the beta-catenin/Wnt pathway, which induced the cell to divide and multiply [25].

Tideglusib was a potent, selective, and irreversible small molecule non-ATP competitive GSK-3 inhibitor for the treatment of Alzheimer's disease and progressive supranuclear palsy [26,27] given the substantial recent literature discussing the involvement of GSK-3 in the molecular pathways of various diseases [28]. The Spanish pharmaceutical business Zeltia group was developing Tideglusib, as an Alzheimer's disease therapy in 2012 [29].

It had also been noted that a biodegradable collagen sponge delivered the medicine to the exposed pulps of rat molar teeth in a unique way in dental tissues [30]. It was claimed that by inducing the production of RD, the medication effectively repaired deep tooth injuries [31].

The MTA had a good dentin bridge development and reduced inflammatory reaction, according to several types of research in people and animals [32]. Based on the findings of earlier investigations, the MTA was currently the standard restorative material and was used in the majority of studies that

compare the effectiveness of novel restorative materials [33].

To compare Tideglusib's effects on dental pulp repair through the stimulation of stem cells to MTA, this study was done because the previous literature could not fulfil all information on the drug's use in dental repair.

2. Patients and methods

Twenty-five adult healthy male rabbits ranging from 3 to 4 months and of mean weight (2.8 ± 0.4) kg were used in this study. The rabbits were housed in the Faculty of Pharmacy, Al-Azhar University, and maintained in a 12-h dark-light cycle, with a humidity of 50 ± 5% and at a temperature of 22 ± 3 °C and good ventilation. The rabbits were fed a soft diet of fresh hay, water, and fresh vegetables. The study was conducted according to the guidelines of the institution's Animal Ethics Committee of Al-Azhar University with the final Code REC-B1-23-01.

2.1. Sample size

A power calculation test was performed, setting an effect size of 0.80, $\alpha = 0.05$, and a power at 80%. The sample size calculation showed a requirement for eight subjects per group. Accordingly, in the present study10 subjects were recruited per group except for the control group, which consisted of five according to the ethical considerations.

2.2. Steps of induced pulp exposure

The rabbits were anesthetized by equal parts of ketamine and xylazine (Nexgen) subcutaneous injection. Pulp exposure was induced by a round carbide bur (FG ¼) attached to a high-speed handpiece with a coolant to avoid heat generation until the dentin was reached, and then the pulp was penetrated using a 30G needle. The pulp exposure was performed on the right and left mandibular first molars.

2.3. Pulp capping procedures

After pulp exposure, the animals were divided randomly into control group I and two experimental groups (II and III):

Control group I: Five molars with normal pulp without pulp exposure for 6 weeks. MTA group II: 10 M with pulp exposure capped with MTA subdivided into group IIa: capping for 4 weeks and group IIb: capping for 6 weeks.

Tideglusib group III:10 M with pulp exposure capped with Kolspon Sterile Collagen Plug Sponge (Tricare company) in association with 50 nM Tideglusib powder (Sigma Aldrich Company) dissolved and diluted in DMSO (dimethyl sulfoxide) (Heiltr Pen USA.Company) [29]. They were subdivided into group IIIa: capping for 4 weeks, group III b: capping for 6 weeks. Glass ionomer cement in powder and liquid form (Micron, Prevest DenPro Company) was used as a sealing material for controlling the mixture consistency to achieve a translucent shade. Analgesic (Meloxicam) was given for the animals until recall as it had appeared beneficial to choose the higher recommended daily dose of 1 mg/kg when using meloxicam for analgesia in rabbits. After decapitation, teeth were extracted after 4 weeks and 6 weeks. Figs 1 and 2 showed Tideglusib drug and Kolospon sponge, respectively.

2.4. Histological Hematoxylin and Eosin stain

The samples that were collected at 4 and 6 weeks were decalcified for 6 weeks in 19% EDTA; the teeth were fixed in 10% neutral buffered formalin (Sigma Aldrich).

They were then dehydrated in ascending concentrations of ethyl alcohol (from 50% to 100%), infiltrated with Xylene (Xylol), and embedded in paraffin wax (Alexandria) blocks. Sections of 8 μm thickness were prepared for staining with Hematoxylin and Eosin (H and E).

2.5. Transmission electron microscopy (TEM)

Tooth pulp tissue was collected according to the experiment time course after injury. The extracted teeth were kept in ice-cold phosphate-buffered saline (PBS). Using a 23-scalpel blade the molars were separated at the crown–root junction, so that the pulp chamber could be visualized. Using a 0.6 mm straight-tip tweezer, the pulp was gently scraped from the pulp chamber. Ultra sections were stained

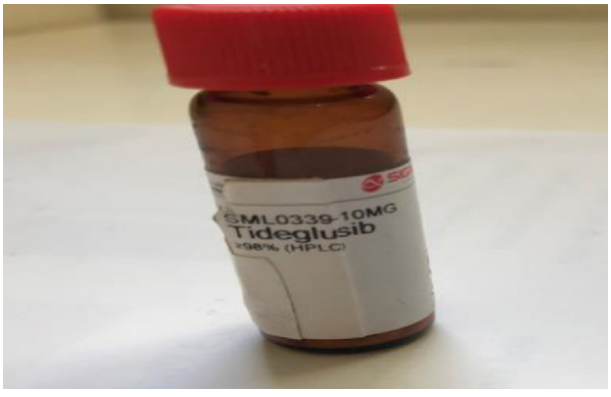

Fig. 1. Tideglusib drug.

Fig. 2. Kolspon collagen sponge.

with uranyl acetate and lead citrate to be examined with TEM (Joel 1000) at the Regional Centre for Mycology and Biotechnology Al-Azhar University.

2.6. Immunohistochemical study using OctA-4 antibody to detect stem cell stimulation in the pulp tissue

Specimens were routinely fixed, dehydrated, and cleared and then paraffin wax was embedded. Paraffin tissue sections were deparaffinized and rehydrated followed by antigen retrieval. The sections then were incubated with a blocking buffer for 1 h, then incubated with the primary antibody, rabbit polyclonal anti-OctA-4 Antibody purchased from Bethyl Laboratories, Inc. Montgomery, Texas, USA).

Detection of bound antibodies was done by the EconoTek HRP Anti-Polyvalent (DAB) kit purchased from ScyTek Laboratories, Inc., Utah, USA as recommended by the manufacturer's instructions. The slides were stained with diaminobenzidine (DAB) chromogen to detect the reaction product (a brown-colored epitope).

2.7. Histomorphometric analysis

Histomorphometric measurements were performed using the 'Optics Image Analyzer Computer System'. The analytical evaluation was done using the image analysis software (Image J,1.41a, NIH, USA). Color thresholding was performed automatically to convert the positive staining into measurement units (pixels) produced by the image analyzer program. The quantitative measurement analysis of immunohistochemical stain depends on the gray value from dark to light stain reaction, which is from 0 up to 10: (0–1 intense, 2–3 strong, 3–4 moderate, 4–7 weak). The data collected from the image analyzer were statistically analyzed.

2.8. Statistical analysis

Statistical analysis was done using SPSS 20; SPSS, Chicago, IL, USA. Paired t-tests and independent t-tests were performed to compare the grade intensity of OctA-4 at 4 weeks and 6 weeks mean scores between the MTA group and Tideglusib group. P values less than or equal to 0.05 were considered significant.

3. Results

3.1. Histological results

3.1.1. Control group I: without pulp exposure

The results of the control group after 6 weeks showed normal zones of the pulpal tissue, where the odontogenic zone was normal with palisading odontoblasts with a normal structure laying in the dentin side of the pulp. The cell-free zone, cell-rich zone, and central pulp core exhibited normal nerve fibers and blood vessels (Fig. 3).

3.1.2. MTA group IIa

In other specimens, the exposure area showed a localized area of RD. The pulpal tissue showed an area of complete pulpal degeneration, multiple engorged blood vessels with blood as well as multiple inflammatory cells. The odontoblastic layer either degenerated or lacked the normal arrangement and detached from the pulpal side wall. Along the pulpal side, layers of RD were observed (Fig. 4).

3.2. MTA group IIb

At the area of the exposure, a layer of RD bridge was observed. The bridge extended and completely closed the exposure area. There was an area of

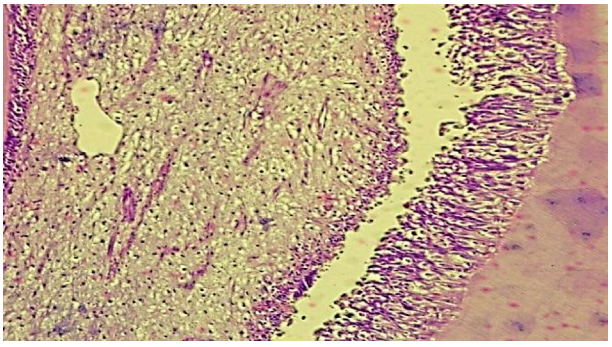

Fig. 3. Photomicrograph of the control group showing normal zones of the pulpal tissue, where the odontogenic zone was normal with palisading odontoblasts with normal structure laying in the dentin side of the pulp, cell-free zone, cell-rich zone, and central pulp core (H and E stain, origin. Mag. ×400).

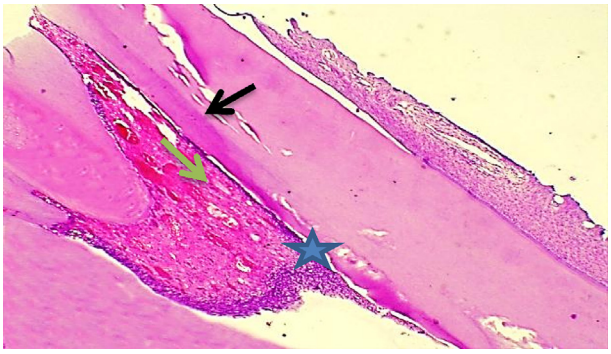

Fig. 4. Photomicrograph of teeth filled with mineral trioxide aggregate IIa, showing a localized area of reparative dentin (green arrow), areas of complete pulpal degeneration (blue stars), multiple engorged blood vessels with blood (black arrow), multiple inflammatory cells, degenerated odontoblasts, odontoblasts detached from pulpal wall lacking normal arrangement (Od), primary dentin (primary D), and reparative dentin (RD) (H and E stain, origin. Mag. ×400).

complete pulpal degeneration underneath the dentin bridge. The remaining pulpal tissue lacks the normal architecture of the pulp (Fig. 5).

Higher magnification of the previous figure showed the formation of a RD bridge at the exposure area. Underneath the dentin bridge, there was an area of complete pulpal degeneration as well as a layer of vacuolated odontoblasts. The remaining pulpal tissue relatively lacks the normal architecture and shows multiple small discrete areas of calcification and oedematous spaces (Fig. 6).

3.3. Tideglusib group IIIa

There was a pronounced amount of RD enclosed minute area of pulpal tissue extended at the exposure area. The remaining pulpal tissue showed high vascularization with multiple engorged blood

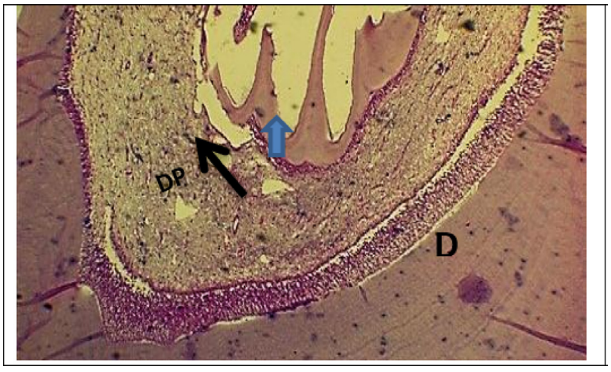

Fig. 5. Photomicrograph of teeth filled with mineral trioxide aggregate IIb showing the formation of dentin bridge at the exposure area of the pulp (blue arrow), areas of pulp degeneration: black arrow, dental pulp (DP); D, dentin, (H and E stain, origin. Mag. ×200).

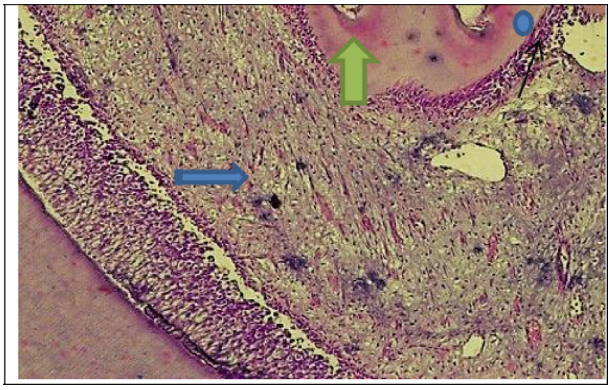

Fig. 6. Higher magnification of the previous photomicrograph of teeth filled with mineral trioxide aggregate IIb showing the formation of reparative dentin bridge (green arrow), areas of degeneration (black arrow), vacuolated odontoblasts (circle), edematous areas (E), small areas of calcification (blue arrow) (H and E stain, origin. Mag. ×400).

vessels with blood. An area of complete pulpal degeneration was noticed. Along the pulpal side wall, a layer of RD was formed (Fig. 7).

In another specimen, complete obliteration of the exposure area by the formation of a huge amount of RD encloses a minute area of remaining pulpal tissue and cells forming an area of osteodentin underneath the exposure area (Fig. 8).

3.4. Tideglusib group IIIb

The details of the photomicrographs are: The exposure area was obliterated by a thick amount of RD which showed different areas of stainabilities. The underneath remaining pulpal tissue showed relatively normal architecture with small discrete areas of calcification. As shown in another specimen, the exposure area occluded with the

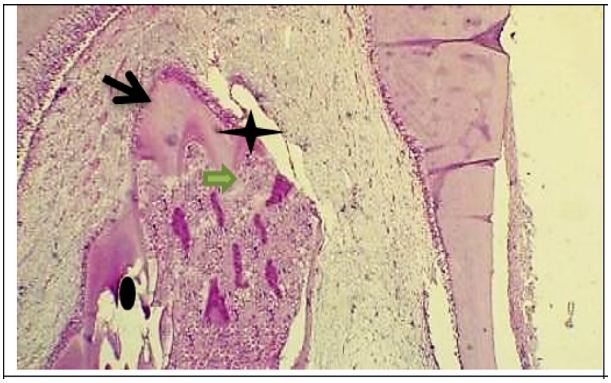

Fig. 7. Photomicrograph of teeth filled with Tideglusib IIIa group showing a pronounced amount of reparative dentin at the exposure area (black arrow), a minute area of pulpal tissue extended at the exposure area (black circle), an area of complete pulp degenerated (black stars), and large area of calcification (green arrow), (H and E stain, origin. Mag. ×400).

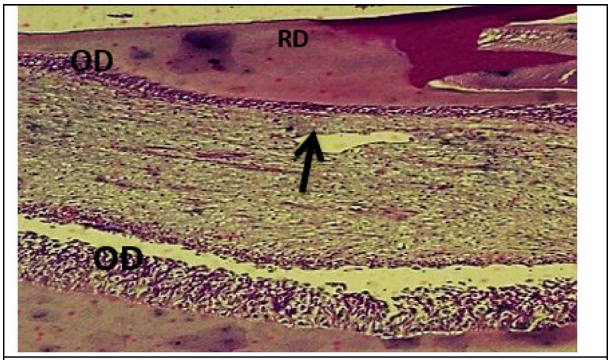

Fig. 8. Higher magnification of other specimens of teeth filled with Tideglusib IIIa group showing a pronounced area of complete pulpal degeneration (black arrow), relatively intact odontoblasts (od), and multiple layers of reparative dentin (RD) (H and E stain, origin. Mag. ×400).

formation of thick layers of RD showing a lesser degree of calcification than the primary dentin and separated from it by a line of demarcation. The remaining enclosed pulpal tissue showed diffuse extravasated RBCs with minute areas of hemorrhage (Figs 9 and 10).

3.5. Immunohistochemical results

In the control group, the immunohistochemical stain of OctA-4 showed a mild cytoplasmic reaction in the sub-odontoblastic area.

In MTA groups IIa and IIb, the immunohistochemical stain of OctA-4 showed diffuse moderate to strong cytoplasmic reaction in the sub-odontoblastic layer and central part (Figs 11 and 12).

Immunohistochemical results of Tideglusib IIa and IIb groups showed expression of OctA-4 in most parts of the pulpal tissue, where group IIIb showed a more intense reaction than that of the 4 weeks group and this was an indication for the presence of stem cells (Figs 13 and 14).

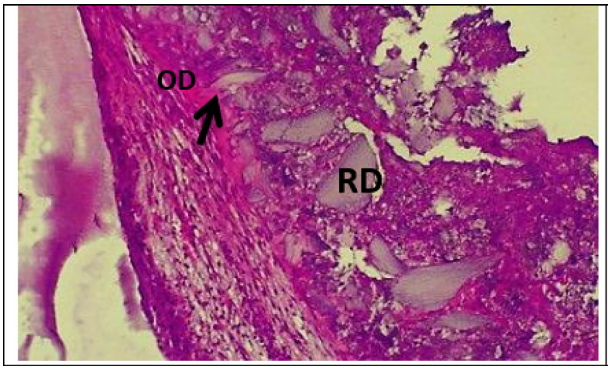

Fig. 9. Photomicrograph of teeth filled with Tideglusib IIIb group showed closing of the exposure site by reparative dentin (RD), hyalinized area of the pulpal tissue (black arrow), irregularly arranged odontoblastic layer (Od) (H and E stain, origin. Mag. ×400).

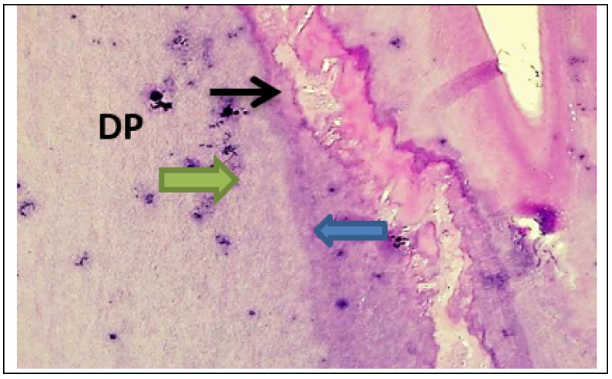

Fig. 10. Photomicrograph of teeth filled with Tideglusib IIIb group showing a thick layer of reparative dentin occluding the pulp space (green arrow), extravasated RBCs with hemorrhage are present in the remaining pulp (black arrow), and diffuse calcification (blue arrow) (H and E stain, origin. Mag. ×400).

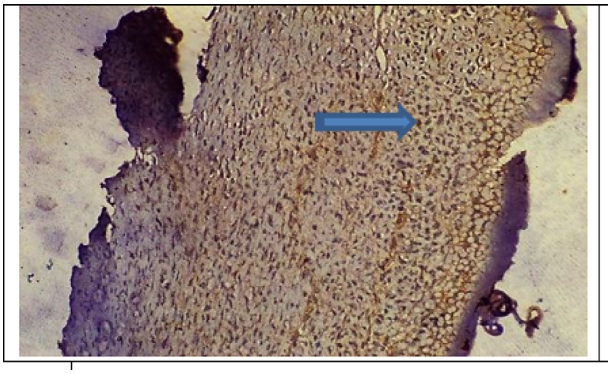

Fig. 11. Photomicrograph of pulp tissue of the mineral trioxide aggregate IIa group showing a moderate diffuse OctA-4 monoclonal antibody immunostaining reaction in the sub-odontoblastic area (blue arrows); color developed by DAB (origin. Mag. ×200).

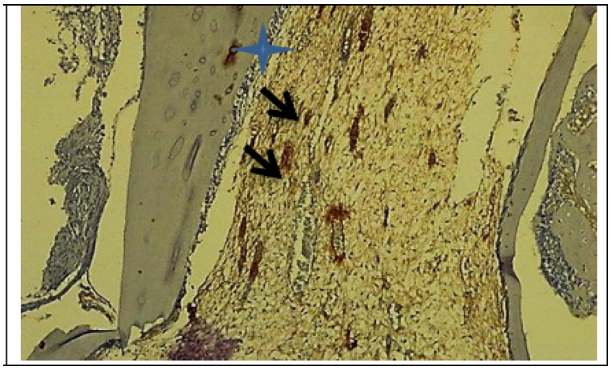

Fig. 12. Photomicrograph of mineral trioxide aggregate IIb group showing moderate to strong diffuse OctA-4 monoclonal antibody immune staining in sub-odontoblastic cells (blue stars), a moderate reaction in endothelial cells of few blood vessels (arrows); color developed by DAB (origin. Mag. ×250).

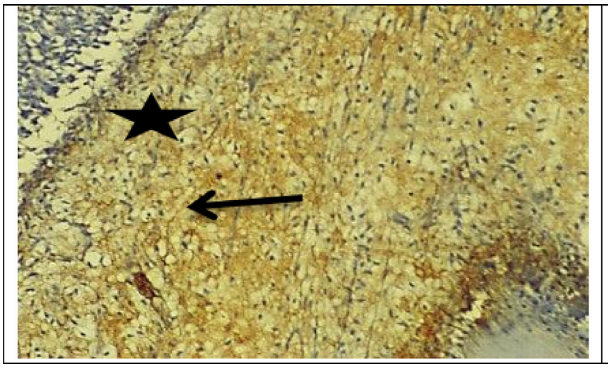

Fig. 13. Photomicrograph of Tideglusib drug group IIIa showing strong granular positive immunoreaction for OctA4 monoclonal antibody in dental pulp cells (black arrows) and diffuse reaction in sub-odontoblastic cells (stars); color developed by DAB (origin. Mag. ×400).

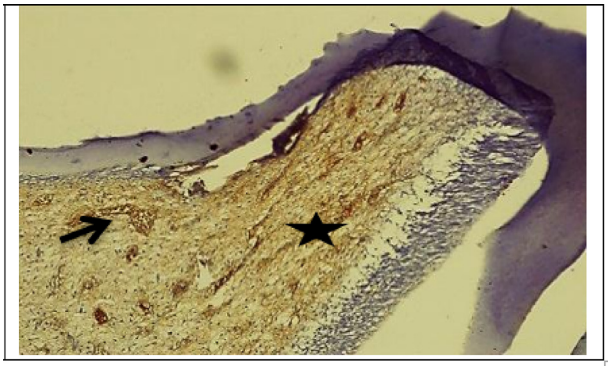

Fig. 14. Photomicrograph of Tideglusib drug group IIIb showing intense OctA-4 monoclonal antibody immunoreaction in the sub-odontoblastic area (star) and around blood capillaries (black arrows), color developed by DAB (origin. Mag. ×250).

3.6. Statistical analysis

The analysis of collected data showed a significant decrease in the positive staining of OctA-4 between the Tideglusib group and the MTA group. The highest mean value of OctA-4 was recorded in Tideglusib IIIa and IIIb groups with only a significant difference in *P* value between the groups in 6 weeks (Figs 15 and 16).

3.7. Transmission electron microscopy (TEM) results

In the control group, ultrastructural results revealed normal odontoblastic cells with open-faced nuclei, well-developed endoplasmic reticulum, and Golgi complex exhibiting normal cell junctions and many electron-dense granules.

In the MTA group, ultrastructural results of MTA IIa and IIb groups showed a photo micrograph with an active odontoblast-like cell with an open-faced

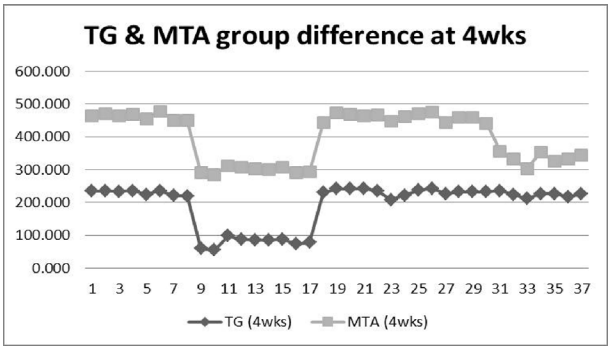

Fig. 15. Linear graph chart illustrates the difference between both groups (TG IIIa and mineral trioxide aggregate IIa).

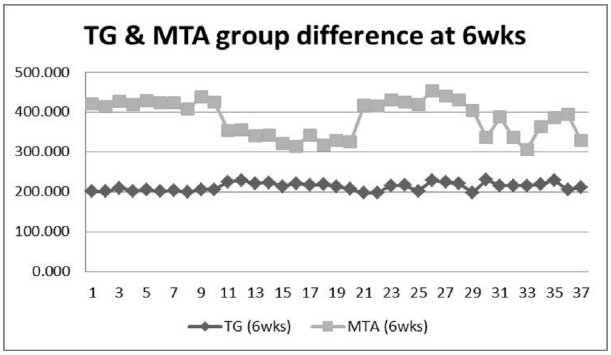

Fig. 16. Linear graph chart illustrates the difference between both groups (TG IIIb and mineral trioxide aggregate IIb).

nucleus and well-developed rough endoplasmic reticulum and Golgi complex but with many vacuolations and less electron-dense granules in the sub-odontoblastic area. It also showed active odontoblast and fibroblast cells with some degenerated fibroblasts. Rounded dense granules were observed within the odontoblastic cells but with fewer numbers compared with the Tideglusib group (Figs. 17 and 18).

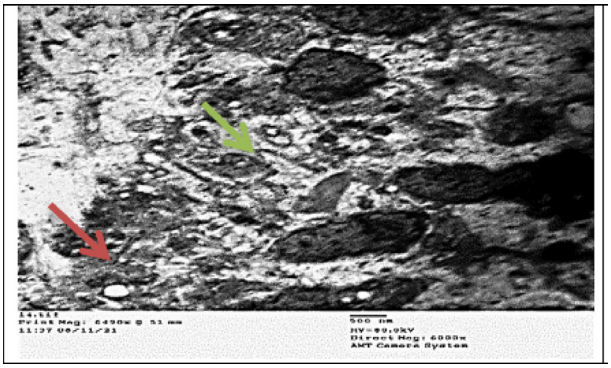

Fig. 17. Photomicrograph of pulp tissue ultrastructure of the mineral trioxide aggregate IIa group showed active odontoblast-like cells with open-faced nucleus with many vacuolations (green arrow) and fewer electron-dense granules (red arrow) in the sub-odontoblastic area (uranyl acetate and lead citrate × 4000).

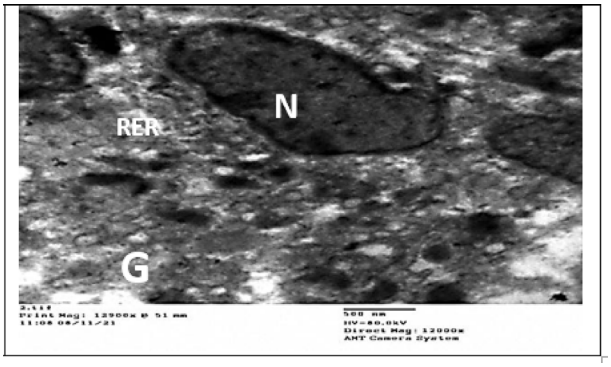

Fig. 18. Photomicrograph of pulp tissue ultrastructure of the mineral trioxide aggregate IIb group showing an odontoblastic cell with an open-faced nucleus (N), well-developed rough endoplasmic reticulum, and Golgi bodies (G) with scattered electron-dense granules and many vacuolizations (uranyl acetate & lead citrate × 12,000).

Ultrastructural results of Tideglusib drug IIIa and IIb groups showed that the odontoblast nucleus had an open-faced nucleus. In addition there was a prominent Golgi complex on the side of the nucleus.

There were numerous rough endoplasmic reticulum and mitochondria scattered throughout the cell. They possessed a junctional complex. Many electron-dense granules were scattered along the cell showing its hyperactivity and its reparative activity.

Another picture showed many fibroblast-like cells with centrally placed nuclei, numerous, well-developed Golgi bodies, and rough endoplasmic reticulum showing proper active protein-forming cells (Figs 19–21).

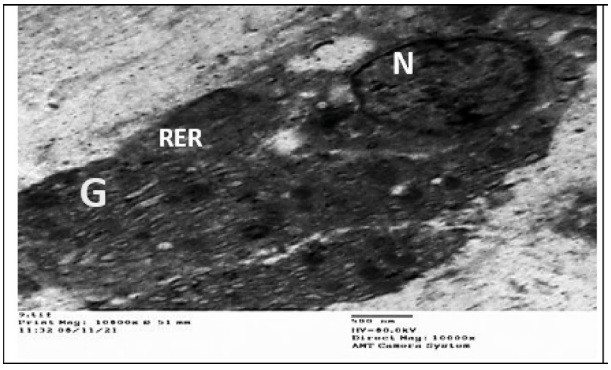

Fig. 19. Photomicrograph of pulp tissue ultrastructure of Tideglusib IIIa showing odontoblastic cell with an open-faced nucleus (N), well-developed rough endoplasmic reticulum, and Golgi bodies (G) with more electron-dense granules (Uranyl acetate and Lead citrate × 10,000).

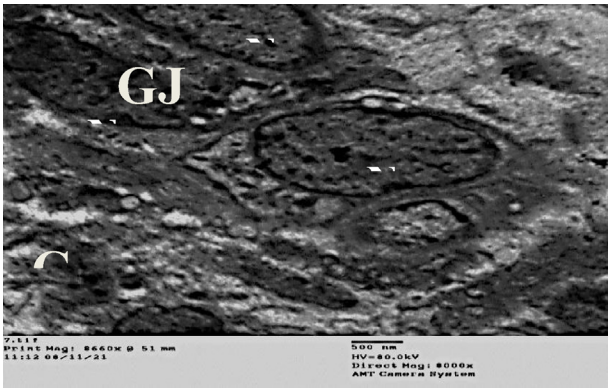

Fig. 21. Photomicrograph of pulp tissue ultrastructure of Tideglusib IIIb group showing many odontoblast-like cells with an open-faced nucleus (N) with proper gap junctions (GJ) and active Golgi body (G) seen (arrow) accompanied by nerve fibers with Schwann covering (uranyl acetate and lead citrate × 8000).

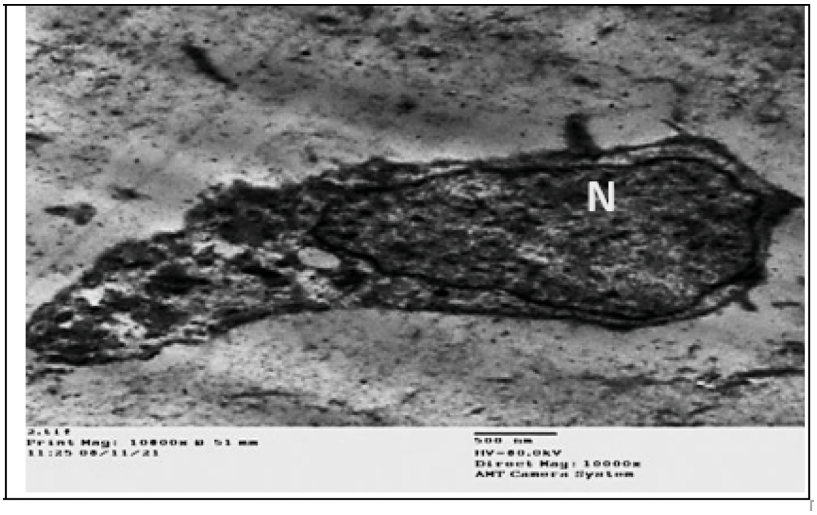

Fig. 20. Photomicrograph of pulp tissue ultrastructure of Tideglusib IIIa group showing a typical fibroblastic cell with its cytoplasmic process and centrally open-faced nucleus (uranyl acetate and lead citrate × 10,000).

4. Discussion

The dental pulp healing process included RD formation after direct pulp capping with any dental material. Wnt/-catenin signaling was activated in response to tissue injury and seems to be essential for tissue repair [34,35].

According to the findings, pharmacological inhibition of GSK3 with specific inhibitors increased the expression of key genes by activating the Wnt/-catenin signaling cascade. The biological efficiency of Tideglusib as a GSK-3 inhibitor in the pulp tissue and dentin bridge development was also assessed using a rabbit model of pulp exposure [36].

Rabbits were chosen as the study's animal model because their teeth are larger than other rodents, making them better suitable for restorative procedures [6]. In addition, many teeth can be selected for each experiment in a single rabbit, minimizing the number of animals required in a single study [33].

MTA was chosen as a positive control because it stimulates RD growth better than conventional materials according to various studies [34,35].

After 4 weeks, no restorative material was seen in the exposed area in the TG group, according to our findings. The biodegradable collagen sponge was used to distribute the Tideglusib medication rapidly, which could be the reason for the lack of the restorative substance. The voids were filled with RD and all of that is assisted by another study, which used a biodegradable collagen sponge to deliver the medicine [37].

Our data showed that the MTA group developed a calcific barrier after 4 weeks, which is consistent with a previous study that used MTA as a capping material [38]. MTA's high success rates in forming a calcific barrier could be due to their high calcium oxide content, which could create Ca $(OH)_2$ when exposed to water [38] $Ca(OH)_2$ is a chemical substance that affects the microvasculature, creating a positive calcific reaction in the pulp tissue and lowering plasma outflow. In animal models, calcite crystal-like structures have been identified near MTA-filled dentinal tubules [38].

All specimens in the Tideglusib group had a full dentin bridge after 4 weeks. Early literature supports the histological findings mentioned above [32]. Others have discovered that the Tideglusib drug promotes Wnt signaling, which could explain how the dentin bridge forms [39].

Furthermore, osteodentin was a type of tertiary dentin seen in the TG group specimens after 4 weeks. This study is backed by the findings of another study, which revealed that during the early phases of RD, odontoblast-like cells are trapped in the dentin bridge construction zone of osteodentin [29].

Moreover, at 4 weeks the MTA IIa group was penetrated by many inflammatory cells. This could be related to the MTA's alkaline pH and calcium ion release, both of which cause the inflammatory response [38].

The increase in vascularity in the TG IIIa group at 4 weeks was a surprising discovery in this study; this finding could be explained by the fact that hard tissue deposition and new tissue growth occurred simultaneously with the formation of new blood vessels [39].

Furthermore, both groups had a high vascular blood supply after 6 weeks, with the TG IIIb group showing an increase in its propensity. This discovered neoangiogenesis indicates effective tissue remodeling and regeneration activities in the pulp [3].

Our immunohistochemical results with OctA-4 [40] marker for stem cell detection revealed intense to strong reactions in the sub-odontontoblastic area and at the central core of the pulp tissue in Tideglusib IIIa and IIIb groups [4].

While in the MTA group was moderate to strong, confirming that Tideglusib could induce stem cell proliferation more than MTA, the presence of intense staining around blood vessels confirmed the presence of neo-angiogenesis [4].

The ultrastructural results of Tideglusib IIIa group showed that odontoblast-like cells in sub-odontoblastic zones with many electron-dense granules were seen scattered along the cell showing its reparative activity.

Another specimen showed a dilated and engorged blood vessel seen near some odontoblastic cells in the sub-odontoblastic zone of the pulp, Also, nerve fibers at the central core of pulp were seen, containing Schwann covering and accompanied by blood vessels which confirmed the vitality of the pulp after capping with Tideglusib.

Both Tideglusib and MTA had good pulpal responses and created a nearly full calcific barrier, according to our data.

4.1. Conclusions

From the previously mentioned results, the following could be concluded:

(1) Small molecule GSK-3 antagonists (Tideglusib drug) delivered through a biodegradable collagen sponge provide an excessive formation of RD of experimentally induced deep dental lesions.
(2) Obliteration of most pulpal exposure sites with tertiary dentin when treated with Tideglusib drug.

(3) Tideglusib can be used as a capping material that works through Wnt signaling which induces stem cell differentiation.

4.2. Recommendations

Based on the results of this study, further studies on Tideglusib in animal models are recommended regarding:

(1) Dose and duration
(2) Technique of application
(3) Pulp vitality test (thermal and electrical).

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Conflicts of interest

There was no conflict of interest.

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