Original Article

Impact of Metformin on Odontoblastic Differentiation and Root Formation During Tooth Development of Diabetic Rat's Offspring

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ABSTRACT

Introduction: Gestational diabetes mellitus (GDM), a clinical class involving pregnant patients with diabetes during pregnancy, negatively impacts fetal development, leading to defects in dental enamel and deciduous teeth abnormalities.

Aim: Evaluating the effect of metformin on odontoblastic differentiation, root formation and tooth development in offspring rats born from diabetic mothers.

Methodology: After induction of pregnancy in 12 adult Sprague-Dawley female albino rats weighing 180-200g and confirmation of day zero of pregnancy, rats were subsequently divided into three groups (N=4 each). Group I (Non-diabetic control): the pregnant female rats were administered citrate buffer solution only. Experimental GDM was induced in groups II and III via injection of a single shot of streptozotocin (STZ) 45 mg/Kg body weight intraperitoneally. Group II was left untreated, Group III was treated with metformin 50mg/kg body weight once daily from the 4th day of pregnancy until birth. Thirty-six offspring rats were obtained (12 from each group). Six offspring from each group were sacrificed at 10 and 21 days after birth. The heads were collected, demineralized and processed, then stained with hematoxylin and eosin, and Nestin immunohistochemical staining. The results were evaluated quantitatively using image analysis followed by one-way ANOVA and post-hoc Tukey statistical analysis.

Results: Diabetes negatively affected the odontogenesis process. Ameloblasts and odontoblasts showed degenerative changes accompanied by delaying in the root formation and eruption. A significant decrease in Nestin expression was noticed in the diabetic group. Treatment with metformin improved the cellular state, and also significantly improved the root length, Nestin expression, and odontoblastic differentiation.

Conclusion: Treatment with metformin ameliorates the adverse effects of gestational diabetes on odontogenesis process, eruption state and odontoblastic differentiation in offspring rats born from diabetic mothers. So, it is recommended that females with gestational diabetes be treated with metformin to protect the tooth development of their offspring.

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Key Words: Gestational diabetes mellitus; metformin; nestin; odontoblasts; tooth development.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a frequent problem linked with pregnancy leading to unfavorable fetal outcomes[1]. The hyperglycemic state lead to altered phenotypic expression during organogenesis of the offspring[2]. Studies showed that GDM causes considerable bone loss and hypomineralization in teeth by affecting the functioning cells and the concentration of the inorganic minerals in the hydroxyapatite structure of dentin and enamel. Therefore, many of the mechanical characteristics in offspring's teeth may be adversely affected. Few studies declared its damaging effect on odontoblasts and dentin formation[3]. The principal treatment strategy for GDM is lifestyle modifications, however the introduction of drugs is taken into consideration[4]. Metformin is considered the initial pharmacological recommendation for the majority of type 2 diabetic patients either alone or in conjunction with other anti-diabetic drugs like insulin[5]. Numerous in*vitro* studies proved that metformin can stimulate the osteogenic development of various mesenchymal stem cells (MSCs)[6]. Metformin can also enhance odontoblast development and mineralization from dental pulp stem cells (DPSCs) which was evident by the expression of odontoblastic markers[7]. Based on the previous knowledge, this work aimed to study if in *vivo* metformin can prevent the adverse effect of GDM on tooth development and odontoblastic differentiation in offspring rats born from diabetic mothers.

METHODOLOGY

Animal care and sample size

The experimental techniques followed a protocol authorized by the Ethical Committee of Faculty of Dentistry, Mansoura University, Egypt. With approval no A 10100221. The standard guides of the care and use of the laboratory animals were followed during handling of the animals.

For induction of pregnancy, 20 adult female and 10 adult male sprague-dawley albino rats weighing 180-200 g were selected. The animals were housed in standard

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conditions at MERC (Medical Experimental Research Center) in Mansoura (Egypt). The animals were allowed to acclimatize for three weeks at a temperature of 26-28°C , standard humidity 65-70% and 12hr dark-light cycle accompanied by free accessibility to a standard soft high-carbohydrate diet, protein feed 23% and water.

Sample Size was calculated using PASS (power analysis and sample size software, version 15, 2017, Kaysville, Utah, USA: NCSS, LLC). To produce six offspring rats per each sacrification time, a total of 36 offspring rats are needed.

Induction of pregnancy and grouping

The female and male rats (at a 2:1 conception rate) were allowed to mate overnight on the evening of the proestrus stage of the estrous cycle. The female rats were free of diabetes mellitus (the glucose serum level should be less than 7.0 mmol/L). The copulation was verified on the following morning by microscopic evaluation of the vaginal smear and the detection of sperms, or by the appearance of a vaginal or mucous plug. It is considered day zero of pregnancy. Subsequent labeling and separation of the pregnant rats was performed. One week following mating, the unpregnant rats were excluded[8,9].

Twelve pregnant female rats were subsequently divided into three groups (four rats each); Group I (Non-diabetic, control): The pregnant rats were given an equal volume of citrate buffer solution only[10]. Group II (Diabetic): The pregnant rats were subjected to induction of GDM on the first day of pregnancy[9]. Group III (Diabetic + Metformin): The pregnant rats were subjected to induction of GDM. After GDM model was successfully established, treatment with metformin 50 mg/kg body weight began from the 4th day of pregnancy until birth (the average gestation time is 21 to 23 days). It was taken once daily, using an oral cannula or gavage[11].

Induction of Gestational Diabetes Mellitus (GDM)

Eight pregnant rats were fasted beginning on the evening of day zero of pregnancy and lasting roughly 12 hours[12]. On the 1st day of pregnancy, they received a single intra-peritoneal injection of freshly prepared streptozotocin (STZ) (Sigma Chemicals Co., St. Louis, MO, USA) dissolved at a dose of 45mg/Kg body weight in 0.1 M cold sodium citrate buffer (pH 4.5)[9]. The buffer should be prepared freshly for immediate use within 5 min. Group I (non-diabetic, control) received an equal amount of citrate buffer[10,12].

On day 3 of pregnancy, 48 h after STZ injection, the diabetic status was identified by measuring the fasting blood glucose level in pregnant female rats using a Mini Glucometer (One Touch Ultra) with a blood drop acquired from a puncture in the tail vein. Diabetic status was confirmed in pregnant female rats when the value of the fasting blood glucose was over 200 mg/dL, as approved by the WHO[13], or blood glucose levels were found ranging from 13.9–22.2 mmol/L[14]. Then, metformin administration begins from day 4 of pregnancy, the next day of diabetes confirmation.

Regrouping After Measurment of Maternal Fasting Glucose level

On day four of pregnancy, weighing of all pregnant females was performed followed by measurement of fasting blood glucose level before initiation of the treatment. Then, the pregnant diabetic female rats having lower level of glucose were positioned in the diabetic untreated group, while those with high blood glucose levels were allocated to metformin group.

Regrouping was essential in avoiding early death of the pregnant diabetic untreated rats because rats with high diabetes rarely live long without therapy[15]. In addition, collecting the extremely diabetic rats in the drug-treated group will augment the effect of metformin[16].

Specimen Processing and Assessment Methods

Thirty-six offspring rats were obtained from three groups (12 from each group). A hand lens was used to examine each fetus for any morphological abnormality. At 10 and 21 days after birth, six rats from each group were euthanized via injection of a mixture of xylazine (5mg/kg) and ketamine (75mg/kg) intraperitoneally at 10 and 21 days after birth[17]. After harvesting the animals, the heads of all three groups were fixed for 48 hours in 10% neutral buffered formalin. Then, they were conveyed to 14% neutral buffered ethylene diamine tetra acetic acid (EDTA) for proper decalcification after convenient washing under running tap water for about 30 min[18]. After decalcification, the specimens were stained with hematoxylin and eosin (H&E) according to Cardiff *et al*. (2014) protocol[19], and anti-Nestin antibody [(MAB353) mono clonal rat-401, Sigma, St. Louis, MO, USA], a specific marker of odontoblast, according to the protocol done by Nakatomi *et al*.[20]. The slides were numbered and examined blindly. Finally, digital morphometry was carried out for the root length and Nestin expression, then results and data were statistically analyzed.

Digital Morphometric Analysis

At the maxillary first molar area ten fields in each group were examined and histological photographs were taken at 100X and 200X, then they digitized with a Toup Tek Toup view ® digital camera (model no.: XCAM1080PHA) mounted on an Olympus® microscope (CX22, Japan).

For root length measurement, analysis of the photographs was performed using Video Test Morphology® software (Russia) having a specific built-in routine for distance calibration. Measurements were performed using free hand line tool for measurement.

For analysis of Nestin expression: photographs at 200X were analyzed using VideoTest Morphology® software (Russia).

Statistical Analysis

IBM-SPSS software was used for data analysis (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Shapiro-Wilk's test was initially used to test normality, normally distributed data were considered if $p>0.050$. Boxplots tested the presence of extreme values. Quantitative data were presented as mean and standard deviation.

The Independent Samples T-test was utilized in comparison of normally distributed quantitative data between two groups (two time periods) and the one-way ANOVA test was utilized to compare normally distributed quantitative data between more than two groups, followed by the post-hoc Tukey test. Considering results statistically significant if the *p-value* was ≤ 0.050.

RESULTS

Hematoxylin and Eosin Results

10 days after birth

The maxillary first molars tooth germs of albino rats of all groups were at the late bell stage of tooth development (Intra Osseous Phase of Eruption). (Figure 1) The control non-diabetic group revealed the normal structure of the maxillary first molar tooth germ with normal cellular appearance with regular arrangement and normal adhesion. (Figures1A,2A,2B) The enamel was absent in the cusp region of some specimens with short ameloblasts found only in this area directly attached to the underlying dentin. (Figure 2A) The root length (dash line) is determined from the cervical end of the enamel till the epithelial diaphragm (ED) (425±15.14). (Figures 4A,6, Table1).

Group II (Diabetic, non-treated group) revealed some evident signs of degeneration of the maxillary first molar tooth germ. The ameloblast and odontoblast cells exhibited some disturbances in their arrangement with loss of homogenous continuity as they were detached in some areas from the underlying enamel and dentin matrices. There was also loss of cell-to-cell adhesion, with empty spaces (vacuoles) and some eosinophilic areas infiltrating the odontoblastic layer. (Figures 1B,2C,2D) Some odontoblastic nuclei appeared crescent shape. (Figure 2D) The length of the formed root was short (43.83±2.23) (Figures 4B,6,Table1).

Metformin treated group exhibited evident signs of normal development of the maxillary first molar tooth germ. Ameloblasts and odontoblasts revealed almost their normal cellular structure, with improvement in their arrangement, attachment, and physiologic adhesion between each other. (Figures 1C,2E,2F) The deposited dentin matrix and predentin showed slightly higher thickness than the diabetic group in the cervical region of the crown (Figure 4). There was an obvious progress in the root development denoted by the increased length of the developing root (409.8 ±22.07) (Figures 4C,6, Table1).

21 days after birth

The control non-diabetic group displayed the normal structure of the tooth germ of the maxillary first molar. Ameloblasts no longer appeared due to the eruption of the developing upper first molar in the oral cavity. The enamel was completely formed and fully mineralized proved by its complete absence after the decalcification leaving enamel space limited by the smooth gingiva. Odontoblasts revealed a pseudostratified columnar arrangement, with their nuclei appearing in more than one level. (Figures 1D,3G,3H) The developing root showed continuous formation, epithelial diaphragm and an adequate normal length (1375.8± 98.85) (Figures 4D,6,Table1).

The diabetic group showed delayed tooth eruption and root formation of the maxillary first molar. Ameloblasts were detected as a part of reduced enamel epithelium surrounding the enamel space. The pseudo stratification of the odontoblasts did not obviously appear, the cells were disorganized, demonstrating signs of degeneration with lack of cell-to-cell adhesion (empty spaces or vacuoles), and some eosinophilic areas and crescent nuclei were observed. (Figures 1E,3I,3J) The root development was delayed as the developing root appeared shorter (165.67 ± 10.09) (Figures 4E,6Table1).

The treated diabetic group demonstrated signs of normal development of the tooth germ of the maxillary first molar on the cellular and eruptive level. The cells were normally arranged with a normal appearance, and the tooth erupted in the oral cavity by merging between the reduced enamel epithelium and the oral epithelium (Figures 1F,3K,3L). The developing root showed continuous formation with adequate normal length (908±13.74) (Figures 4F,6, Table1).

Immunohistochemical Results (Nestin Expression)

10 days after birth

Nestin showed a cytoplasmic immunoreactivity observed in odontoblast cells, odontoblastic processes penetrating dentin matrix tissue, and in developing pulp tissue, mainly the subodontoblastic layer. The normal mean % area of Nestin expression was 14.138±0.05. The diabetic group exhibited a significant fall in Nestin expression in comparison to the control group, with a mean % area of Nestin expression 5.09± 0.05. After treatment with metformin, the expression started to rise reaching 11.45± 0.13 (Figures 5,7, Table 2).

21 days after birth

The control group showed an increased Nestin expression after 21 days with a mean % area of 22.873±0.06, while the diabetic group showed a significant decrease in Nestin expression compared to both the control group and the same group at 10 days with a mean % area of 3.823±0.06. A significant increase in Nestin expression was noticed in the metformin treated group in comparison to the diabetic group with a mean % area of Nestin expression of 18.402± 0.013 (Figure 5,7, Table 2).

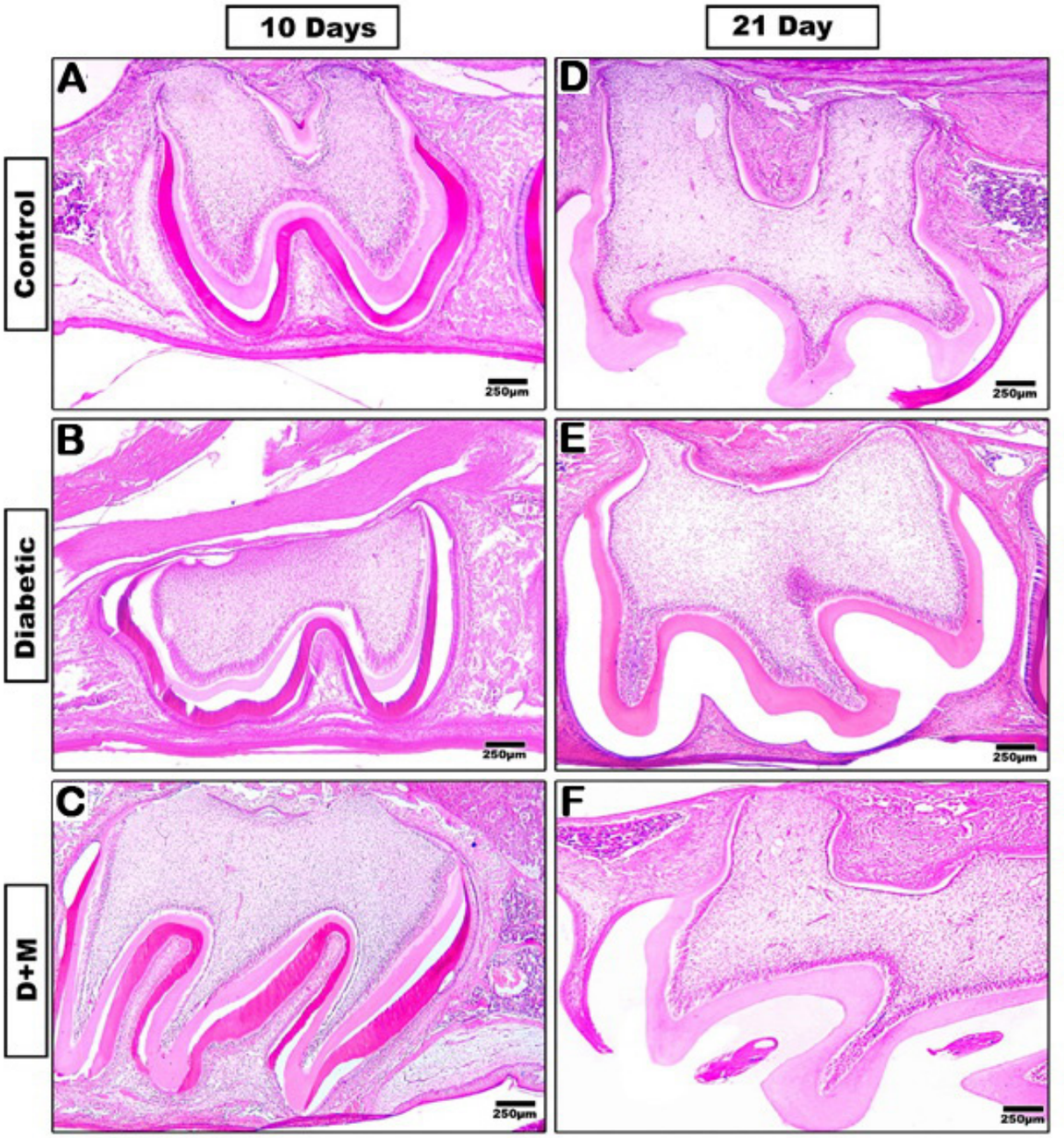

Fig. 1: Photomicrograph showing the eruption stage in the study groups at different periods. All the teeth germs at 10 days were not erupted (A, B&C). After 21 days the teeth germs of the control and treated groups were erupted (D&F), while the diabetic group was not erupted (E). (H&E X40, Bar = 250 µm)

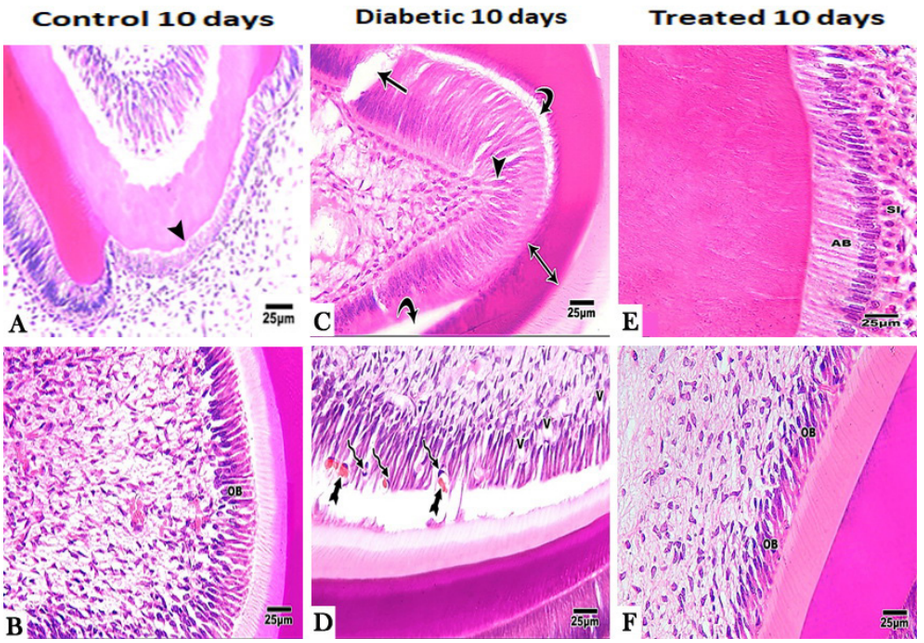

Fig. 2: Photomicrograph showing the ameloblast and odontoblast cells of the study groups at 10 days after birth. The ameloblasts were normally arranged on the enamel of control group with localized shortening (arrow head) and abscence of enamel on the cusp tip (A). Normally arranged odontoblast cells (OB) (B). Ameloblasts of the diabetic group with elliptical nuclei (arrow head), loss of adhesion (arrow), and some area of detachment from the underlying enamel matrix (curved arrows) (C). Detached odontoblasts appeared with eosinophilic areas (tailed arrows), vacuoles (v), and crescent shape nuclei (wavy arrows) (D). Ameloblasts (AB) of the treated group were arranged on a thick enamel matrix layer in a homogenous continuous row (E). Odontoblast cells with normal appearance and arrangement (F). (H&E X400, Bar = 25 µm)

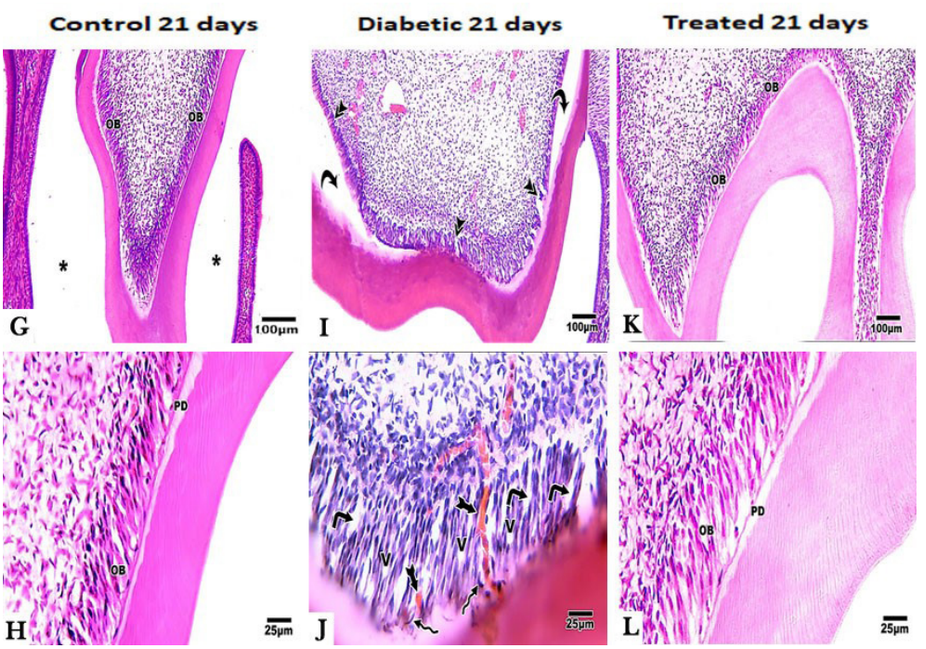

Fig. 3: Photomicrograph showing the odontoblast cells of the study groups at 21 days after birth. Odontoblasts (OB) of the control group were arranged in a single homogenous layer with good adhesion to the predentin (PD) (G&H). Disorganization of the odontoblasts in the diabetic group with discontinuity and tearing (double arrow heads), detachment from underlying dentin (curved arrows), separation between the cells (corner arrows), vacuoles (v), some esinophillic areas (tailed arrows), and crescent nuclei (wavy arrows) (I&J). Odontoblasts of the treated group were arranged in a single homogenous layer attached to the underlying predentin (PD) with minimal vacuolization and elongated nuclei at the proximal ends (K&L). (H&E X100, Bar = 100 µm) & (H&EX400, Bar= 25 µm)

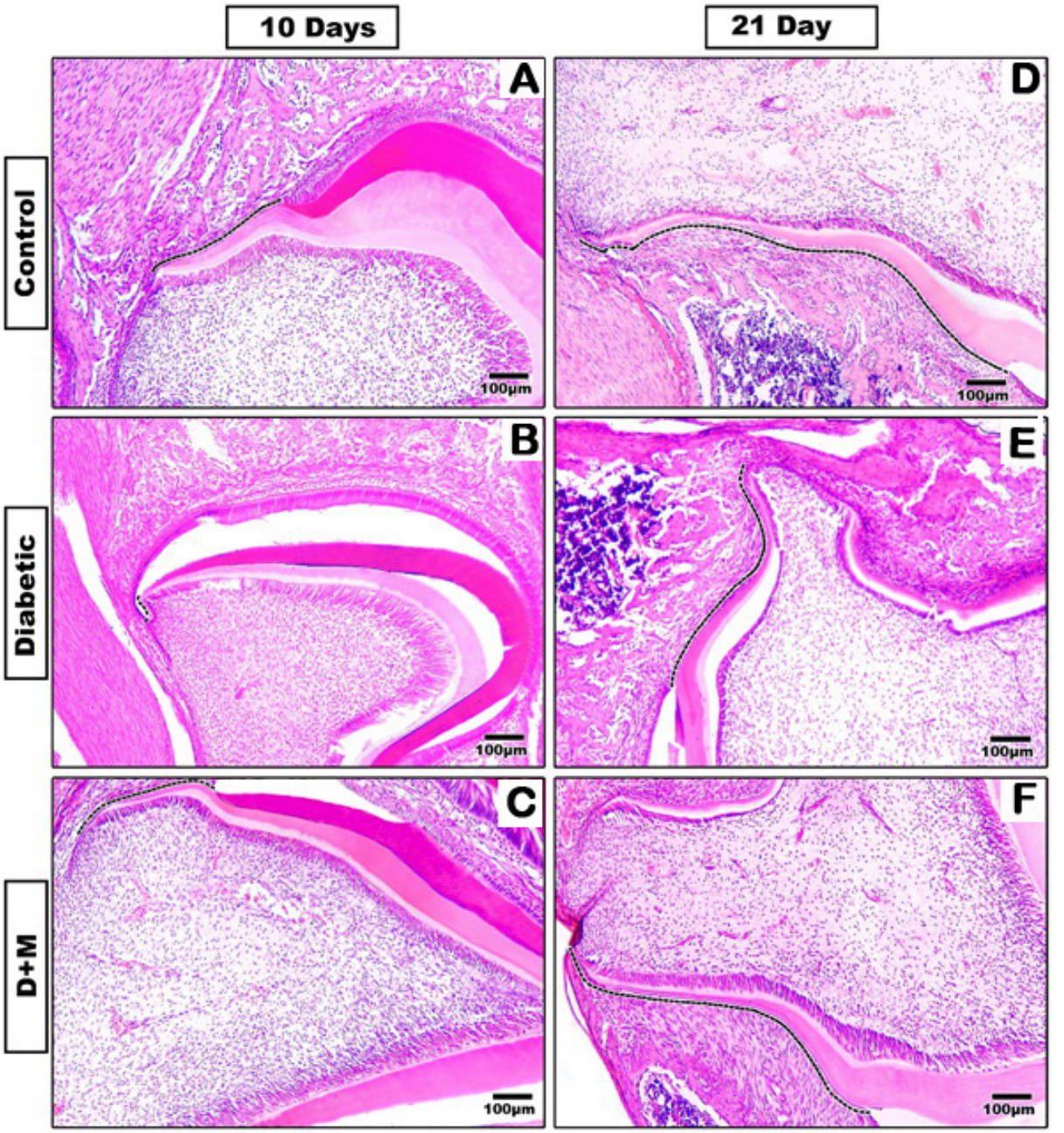

Fig. 4: Photomicrograph showing the length of the developing root (dash line) in different study groups at different periods. Deficient root length of the diabetic group in both tested periods (B&E) in comparison with the control (A&D) and treated group (C&F). (H&E X100, Bar= 100 µm)

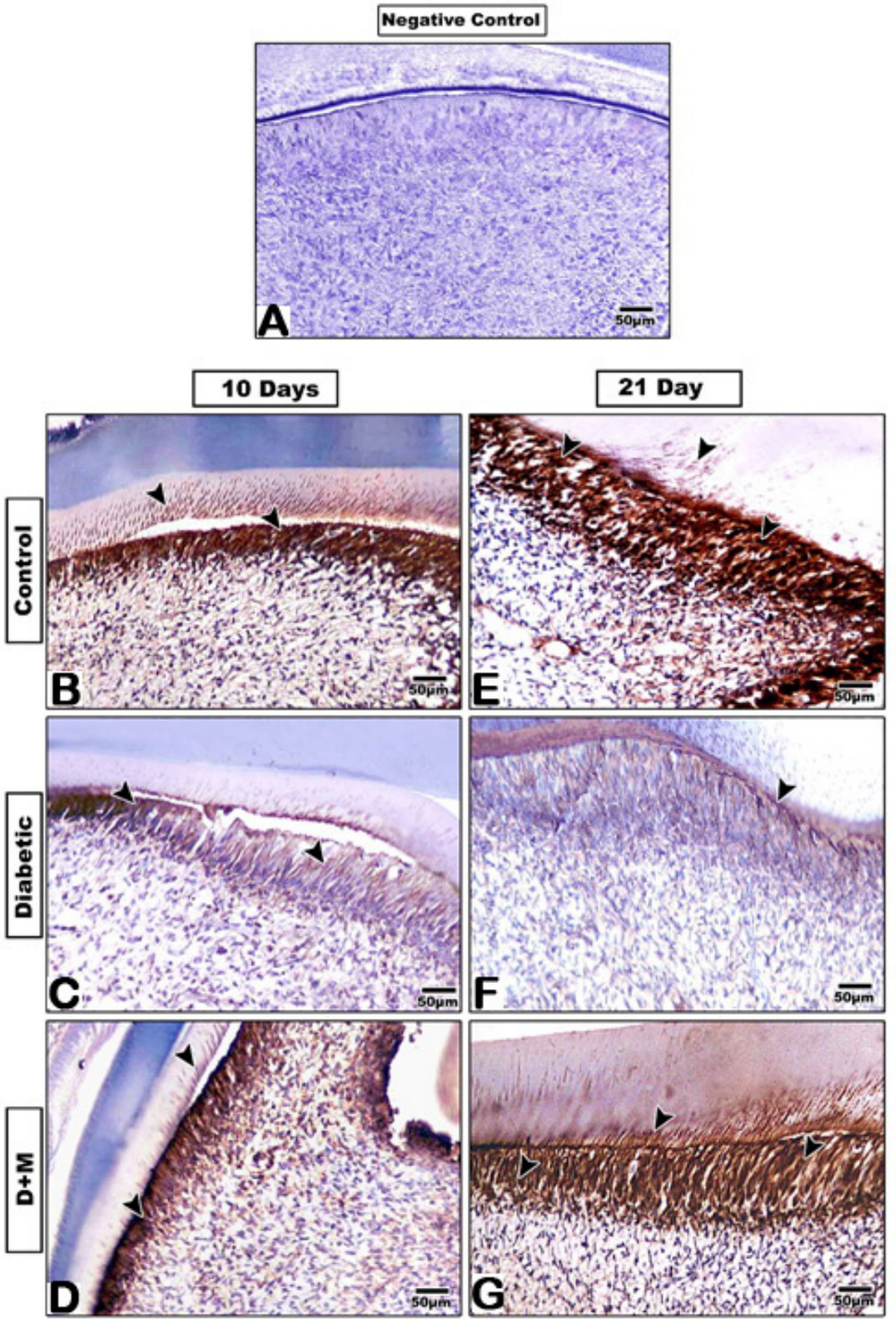

Fig. 5: Photomicrograph showing Nestin expression in all study groups at different periods. Negative control slide of anti Nestin (A). Nestin expression in odontoblastic cell layer with their processes infiltrating dentin matrix tissue (arrow heads) (B, E, D&G). Sever deficiency of Nestin expression in the diabetic group (C&F) in comparison with the control group (B&E) and the treated group (D&G). (Anti-Nestin X200, Bar= 50 µm)

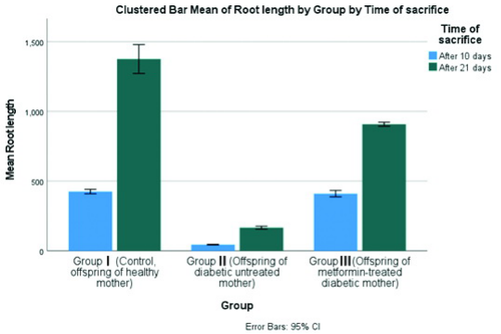

Fig. 6: Clustered bar chart for mean of Root Length in different groups at different periods of time.

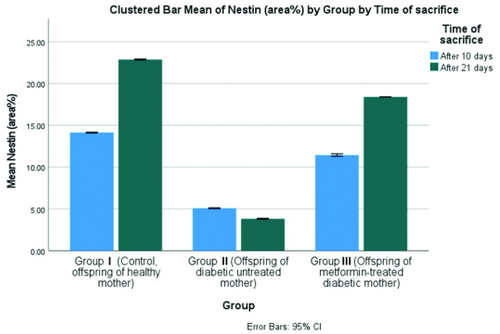

Fig. 7: Clustered bar chart for mean of Nestin positively stained area % for different groups at different periods of time.

Table 1: Comparison of root length in the three groups and two time periods.

Time period	Group						*P2-value*
	Control		Diabetic		Treated diabetic		
	Mean	SD	Mean	SD	Mean	SD	
10-days	425.0	15.14	43.83	2.23	409.8	22.07	<.001*
21-days	1375.8	98.85	165.67	10.09	908.00	13.74	<.001*
P1-value	<.001		<.001		<.001		

Notes: The test of significance is one-way ANOVA (*P2-value*) and independent-samples t-test (*P1-value*).

Table 2: Nestin expression in the three groups and two time periods.

Time period	Group						*P2-value*
	Control		Diabetic		Treated diabetic		
	Mean	SD	Mean	SD	Mean	SD	
10-days	14.14	0.05	5.09	0.05	11.45	0.13	<.001
21-days	22.87	0.06	3.82	0.06	18.40	0.13	<.001
P1-value	<.001		<.001		<.001		

Notes: The test of significance is one-way ANOVA (*P2-value*) and independent-samples t-test (*P1-value*).

DISCUSSION

Due to the consistent correlation between diabetes and pregnancy, a clinical class known as gestational diabetes has been identified.This class comprises pregnant patients whose diabetes begins during the gestational period[21]. Pregnancy-related hyperglycemia is known to negatively impact fetal development, raising the possibility of defects in the dental enamel organ at this time and leading to abnormalities in the deciduous teeth of children born to diabetic mothers[22].

Several drugs could be administered for experimental induction of diabetes like alloxan and STZ, or it could be induced surgically through pancreatectomy. Streptozotocin was utilized to induce diabetes because it possessed characteristics similar to those observed in human cases of diabetes, as well as its broad range of doses with the capacity to be administered in multiple low doses, and its very noticeable effect following intraperitoneal administration[23].

The delivered dose of 45mg/kg was documented in a study to evaluate the influence of okra extract on GDM rats in which Tian *et al*. (2015) employed STZ in the production of GDM. They explained that in adult female rats, a single intraperitoneal dosage of 40 to 60 mg/kg body weight is the most often utilized dose for inducing diabetes mellitus[9]. Moreover, if the dosage is less than 40 mg/kg, it may be ineffective in induction of diabetes mellitus. For optimization of the results, STZ was preserved in a cold store (2-8°C) away from light with immediate preparation before injection, within less than 5 minutes. This is due to the instability of the solution and its reduced ability to induce diabetes if it is not used fresh[24].

Over the past decades, insulin was the drug of choice for treating diabetes. However, several new oral hypoglycemic agents have been established. They have exceptional mechanisms of action that could be directed toward a definite organ[25]. Recent studies proved that metformin can provoke odontoblast differentiation and mineralization in *vitro*, in addition to having an obvious effect in treating

GDM without the production of toxicity signs such as hypoglycemia, renal toxicity and liver toxicity[26,27]. So, it was the drug of choice for our in *vivo* study to evaluate the effect of metformin on tooth development of offspring albino rats born from diabetic mothers.

The50mg/kg metformin was the dose of choice as all concentrations below this value couldn't affect odontoblast cell proliferation, as stated by Qin *et al*. (2018) who used metformin in stimulating the differentiation of dental pulp cells into odontoblasts[11].

The developing rat molars provide an admirable model for studying epithelial morphogenesis. It has many advantages including simplicity in structure analysis which is almost similar to the human molar as they have roots, decreasing in size from before backward, and they are not of persistent growth[28].

The euthanization of the rats at 10 days after birth was based on the fact that at this time, nearly all dental tissues were formed while being under the impact of maternal hyperglycemic status and began the calcification process, as reported by Silva-Sousa *et al* (2003)[29].

The control group at 10 and 21 days after birth showed the teeth germs of maxillary first molars of rats' offspring in the intra-osseous (late bell stage) and extra osseous phase of the eruption, respectively with the normal cellular structure of odontoblasts and ameloblasts. These cells were arranged on a layer of dentin and enamel matrices of adequate thickness. The developing root exhibited normal development and a suitable length measured by histomorphometric analysis. These results were in agreement with Sanchez *et al*. (2020) who conducted a study to investigate the impact of exposure to chromium on dental tissues at different stages of tooth eruption[30].

The diabetic group showed observable signs of tissue degeneration including disruption of the cellular integrity and continuity either in the ameloblasts or the odontoblasts cells with subsequent detachment of the underlying structures. The nuclei showed signs of degeneration as crescent shaped with vacuolization. A shorter length of the developing root was noticed during both periods. In addition, after 21 days, the teeth germs did not erupt into the oral cavity. These results could be explained by Chen *et al*. (2017), who declared that hyperglycemia exhibits various effects on the odontogenesis process in offspring tooth germ. It affects both the proliferation and apoptosis of dental mesenchymal and epithelial cell via enhancement of apoptosis and suppression of odontoblast cells proliferation through (TLR4/NF- κB) signaling pathway[31], which is a pathway activated by maternal diabetes and crucially important in impairing embryonic development[32]. El-Ghawet *et al*. (2019) noticed also the decreased dentin thickness and the damage of some odontoblast cells in the offspring born from diabetic mothers. Widened vacuoles and eosinophilia between the odontoblasts were also detected by H&E. These changes were explained by the increased intensity of caspase immunohistochemical expression denoting increased cellular apoptosis[33]. The damaging effect of diabetes on odontogenesis of the rat offspring could be attributed also to the occurred hyperglycemia during pregnancy that produced a defect in fetal β cells of the pancreas, and consecutively, a reduction in the level of insulin in fetuses, which led to a decline of the glucose transporter 1 (GLUT1) and glucose transporter 4(GLUT4) receptors[34]. The GLUT1 has a role in tooth growth and development as it is a protein required for the transportation of glucose as primary nutrition for the tooth germ of the developing fetus. Another explanation was due to the rise of oxidative stress as a result of the hyperglycemic state that leads to interruption of normal cellular function and deterioration of cellular lipid, protein and DNA[35].

Treatment with metformin showed an improvement in ameloblasts and odontoblasts cellular integrity and arrangement, in addition to improvement in the root length and eruption state. Qotrunnada *et al*. (2021) had similar results since they found that offspring rats born from metformin treated diabetic mothers had accelerated tooth eruption with normal tooth growth and development. The teeth had entered the stage of apposition and calcification with the formation of HERS, and then the eruption stage, compared to offspring rats born from untreated mothers which were still in the late bell stage with no eruption. This may be attributed to metformin ability to improve blood glucose levels and insulin sensitivity during the period of development[36].

In order to detect the influence of metformin on odontoblast cells and dentin regeneration, many in *vitro* studies have been done. They concluded that it would be difficult for the metformin to reach, in a systemic manner, the dental pulp stem cells (DPSCs) residing inside the tooth pulp enclosed by tooth structures[37]. However, in the present work, metformin proved for the first time its obvious systemic effect on odontoblast cell differentiation in addition to the other cells of the enamel organ of upper maxillary first molar born to diabetic pregnant mothers.

Staining with Nestin, a specific marker of odontoblasts[38], proved that diabetes significantly decreases the amount of Nestin expression than the control group. Meanwhile, metformin treatment reversed the deleterious effect on odontoblastic differentiation and significantly increased the Nestin expression approximating the normal level in the control group. Moreover, there was a statistically significant increase in Nestin expression between the two tested periods (10, and 21 days) in the control and metformin groups, while in the diabetic group there was a statistically significant decrease in 21 days vs. 10 days.

This effect may be attributed to the significant upregulation of DSPP gene expression levels (important for specifying odontoblastic differentiation), DMP1, COL1 (an essential protein in dentin formation and repair), SP7

and RUNX2 (key factors essential for the proliferation and differentiation of odontoblasts)[7]. Other studies explained the metformin effect in the enhancement of odontoblastic differentiation of DPCs via the triggering and stimulation of the AMPK signaling pathway[11].

CONCLUSION

This study demonstrated that metformin can improve the degenerative effects of maternal gestational diabetes on offspring's odontogenic cellular differentiation, root formation and tooth eruption. It could also enhance Nestin odontoblastic marker expression. These results suggest that metformin may play an important pharmacological role in triggering odontoblastic differentiation. Therefore, it was advised that any pregnant woman with gestational diabetes take metformin to prevent adverse effects in development of her unborn child's teeth.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير الميتفورمين علي تمايز الخلايا المكونة للعاج وتكوين الجذر أثناء نموأسنان ذرية الفئران المصابة بداء السكرى

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مقدمة : سكري الحمل هو فئة سريرية تشمل المرضى الحوامل المصابات بالسكري أثناء الحمل،ويؤثر سلبًا على نمو الجنين، مما يؤدي إلى عيوب في مينا الأسنان وتشوهات الأسنان اللبنية.
الهدف: أجريت هذه الدراسة لتقييم تأثير الميتفورمين على تطور نمو الأسنان في ذرية الفئران المصابة بداء السكري.
منهجية البحث: تم تحفيز الحمل في ١٢ فأرة بيضاء بالغة، يتراوح وزنها بين ١٨٠-٢٠٠ جرام، و بعد تأكيد اليوم الاول من الحمل، تم تقسيم إناث الفئران الحوامل إلى ثلاث مجموعات (العدد =٤ لكل منها) كالتالي:
المجموعة الأولى :(سليمة بدون مرض السكري): أُعطت محلول السترات المتعادل فقط .
المجموعة الثانية: تم تحفيز مرض سكري الحمل في هذه الفئران في اليوم الاول من الحمل و تركت بدون علاج.
المجموعة الثالثة: تم تحفيز مرض سكري الحمل في هذه الفئران وعُولجت بالميتفورمين، بجرعة ٥٠ ملغم/كغم من وزن الجسم - مرة واحدة يومياً- بدءاً من اليوم الرابع من الحمل حتى الولادة.
تم تحفيز داء سكري الحمل في المجموعة الثانية والثالثة، عن طريق حقن الستربتوزوتوسين، بمقدار ٤٥ملغم/كغم من وزن الجسم داخل الصفاق و التأكد من ارتفاع سكر الدم إلى أكثر من ٢٠٠ملجم/ديسيليتر.
التقييم الهستولوجي و الهستوكيميائي المناعي و الإحصائي: بعد اكتمال الحمل و الولادة، تم الحصول على ستة وثلاثين فأراً مولوداً من المجموعات الثلاث (١٢ من كل مجموعة)، و تم التضحية بستة فئران من كل مجموعة بعد ١٠ أيام من الولادة، وستة آخرين بعد ٢١ يومًا. بعد أخذ عينات الرؤوس و تطريتها، وضعت داخل مكعبات من الشمع، و قطعت على هيئة شرائح لكي تصبغ بالصبغات التالية:

- صبغة الهيماتوكسيلين والأيوسين .
- صبغة نيستين الكيميائية المناعية للكشف عن مدي تمايزالخلايا المولده للعاج.

ثم خضعت النتائج لتحليل الصور والتقييم الكمي، متبوعًا بالتحليل الإحصائي باستخدام اختبار ANOVA أحادي الاتجاه متبوعًا باختبار توكي اللاحق.
النتائج: أظهرت النتائج أن مرض السكري يؤثر سلباً على عملية تكوين الأسنان، في الأجنة المولودة لأمهات مصابة بسكري الحمل، حيث لوحظت تغيرات انتكاسية في خلايا تكوين المينا والخلايا المكونه للعاج ، مصحوبة بتأخر في تكوين الجذر ومراحل ظهور الاسنان. وقد لوحظ انخفاض كبير في صباغة النيستين المناعية في مجموعة مرضى السكري.
أما نتائج المجموعة الثالثة المعالجة بالميتفورمين ، فقد أظهرت حماية خلايا تكوين الأسنان من التغيرات السلبية التي ظهرت في المجموعة الثانية بسبب مرض السكري ، كما أظهرت أيضًا نمو طبيعي لجذور الأسنان وزيادة في صبغة النيستين المناعية الذي يعبر عن تمايز طبيعي للخلايا المكونة للعاج.
خلاصة البحث: اعتمادا على النتائج السابقة يمكننا استنتاج أن العلاج بالميتفورمين يخفف من التأثيرات الضارة لمرض سكري الحمل على تمايز الخلايا المكونة للعاج،والنمو الطبيعي لجذور الأسنان ،وعملية تكوين الأسنان و مراحل ظهورها في ذرية الفئران البيضاء المولودة من الأمهات المصابة بالمرض.
وبالتالي هذه النتائج تقترح ان الميتفورمين ربما يلعب دورًا دوائيًا مهما في تحفيز التمايز الطبيعي لخلايا العصب السنية.