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ENAMEL SURFACE ALTERATIONS IN MOLARS OF YOUNG RATS OF DIABETIC MOTHERS

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ABSTRACT

Objective: Enamel defects are remarkable problems frequently observed in offspring of diabetic women. So, the goal of the current work was determination of enamel surface alterations in molars of young rats born to diabetic mothers.

Material and Methods: 16 heathy adult female albino rats were equally divided into two groups. Induction of diabetes was done in one group by alloxan and the other group act as control group. Four adult male rats were used for fertilization of both groups. All off springs were sacrificed with an overdose of ether at 6 weeks after birth. SEM examination of the occlusal surfaces of the mandibular first molar teeth was done using environmental scanning electron microscope. Teeth were examined for chemical characterization using Electron Dispersive Analytical X-ray (EDAX).

Results: Enamel revealed multiple ultrastructural alterations in diabetic group. By EDAX examination, there was a decrease in the content of calcium and phosphorous in diabetic group as compared with control, altering the calcium to phosphorus ratio.

Conclusion: Maternal diabetes had a detrimental influence on the function of ameloblasts in laying down enamel inducing defective enamel.

Keywords: Maternal Diabetes; Enamel; Ultrastructural Changes; EDAX.

INTRODUCTION

Diabetes is a diversified category of disorders caused by a prorated or absolute insulin deficiency which leads to disturbances of carbohydrate, lipid and protein metabolism[1]. It is very malicious in character and has strong associations with multiple comorbid conditions. Deficiency of insulin production, insulin malfunction or scarcity of insulin receptor responsiveness at target organs lead to hyperglycemia which is considered as the primary feature of diabetes [2-4]. Depending on its signs and symptoms, diabetes can be categorized into three broad categories, namely type 1, type 2 and gestational diabetes that occur solely in pregnant women [5].

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The hyperglycemic state in gestational diabetes has known as mischievous to development of fetus, so danger of disorders at birth increases dramatically[6,7]. Although in recent years, hyperglycemia in gestational diabetes or diabetes mellitus has controlled decreasing perinatal comorbidity and rate of mortality, birth flaws have continued and are at present became the significant reason of morbidness in infants of diabetic mothers[8]. In poorly controlled diabetic patients, there are multiple and devastating oral complications which include an increased susceptibility to different types of infections as viral, bacterial and fungal infections (oral candidiasis), xerostomia (dry mouth), increased liability for risky dental caries, improper wound healing, gingivitis, periodontal disease, periapical abscesses, taste deterioration and burning mouth syndrome[9,10].

Hypoplastic, malformed or defective enamel has been reported in deciduous teeth of children of diabetic mothers [11]. Although however, there is a shortage of investigations on the etiology of its pathogenesis and morphology in human and experimental animals. Therefore, this study was suggested to recognize and describe the morphology of enamel defects through using environmental scanning electron microscope with chemical characterization using EDAX in the mandibular first molar of rats born to diabetic mothers.

MATERIAL AND METHODS

Animals: 16 heathy adult female albino rats weight 200-250 gm with average age of 4-5 months were utilized in this experiment. Other four adult male albino rats were utilized for breeding. The rats were obtained and housed in the animal house at the Institute of Ophthalmology, Cairo University. All rats fed standard commercial diet and drinking water in an air conditioned room. Rats were divided into two equal groups. One group was used as control, other group was subjected to induction of diabetes (Diabetic group).

Induction of diabetic mellitus: Induction of diabetes was done in eight animals (Diabetic group) through a single dose of alloxan 150gm/kg intraperitoneally injected. Sigma Chemical Co. (St Louis, MO, USA) dissolved in a physiological saline (0.9% sodium chloride) solution [12], a saline injection was done only for control group. After 18 hours of fasting period, both groups were injected (60–70 mg/dl blood glucose). The induction of diabetes mellitus was confirmed by a blood glucose concentration greater than 400 mg/dl, determined 2 days following injection of alloxan. During the period of this work, commercial kits were used for monitoring the blood glucose level on a daily basis through carrying out a tiny puncture in tail of the rats. This method is rapid and noninvasive with a negligible stress. Blood glucose level of diabetic group was between 400 and 600 mg/dL.

Experimental design

Four female and one male of both control and diabetic groups were caged separately. Every morning, examination of vaginal smear was done for confirmation of fertilization. The presence of vaginal plug was designated as day zero of gestation. After confirmed breeding, female rats were caged separately. Twenty Male off springs from both groups were used. Ten off springs of control mothers were used as control group and other ten off springs of diabetic mothers were used as maternal diabetic group. All off springs were sacrificed with an overdose of ether at 6 weeks after birth.

Scanning electron microscopic examination and EDAX

Fixation of the removed mandibles were done in 70% Ethyl alcohol. After that , soft tissues were carefully removed. Jaw segments occupied by the first molars were sectioned out using a diamond disc. SEM examination of the occlusal surfaces of the first molar teeth was done using environmental scanning electron microscope (Quanta 200) in the Scanning Electron Microscopic Unit of Research

Center of Monuments. Ministry of Monuments. Cairo, Egypt. Teeth were also examined for chemical characterization using Electron Dispersive Analytical X-ray (EDAX). Weight percent of surface levels of calcium (Ca) and phosphorus (P) were calculated using EDAX. Each tooth was irradiated at the center of its occlusal aspect. Changes in Ca and P percentage levels were recorded and the Ca/P ratio was calculated.

RESULTS

Control group

SEM examination of mandibular first molar teeth of control group showed that, the occlusal surface is formed of a group of cusps arranged in two rows buccal and lingual. There are three buccal cusps B1, B2, B3 and three lingual cusps L1, L2 and L3. There is additional distal cusp at the distal aspect. The cusps are separated by deep grooves traversing buccolingually G1 G2 and G3 (Fig 1). The occlusal aspect of each cusp is covered by enamel at its margins only while the remaining part of the cusps is characterized by presence of tongue like facet of enamel free areas (EFA) that covered by dentine only. The occlusal surface appeared intact and smooth. The enamel free areas were smooth without any cracks (Fig 1&2). All other aspects of the molars are covered by smooth and homogenous enamel surface (Fig 3).

Diabetic group

SEM examination of mandibular first molar teeth of diabetic group revealed severe erosion and defective enamel surface of buccal, lingual and distal cusps (Fig. 4). The sever erosion resulting in complete loss of enamel in some areas of the cusps exposing the underlying dentine (Fig. 5). Higher magnification showed erosion of the enamel rods (Fig. 6). Some cusps revealed cracking ranging from mild to deep cracks extending along the occlusal surface within the enamel as well as enamel free areas (Figs. 7 & 8).

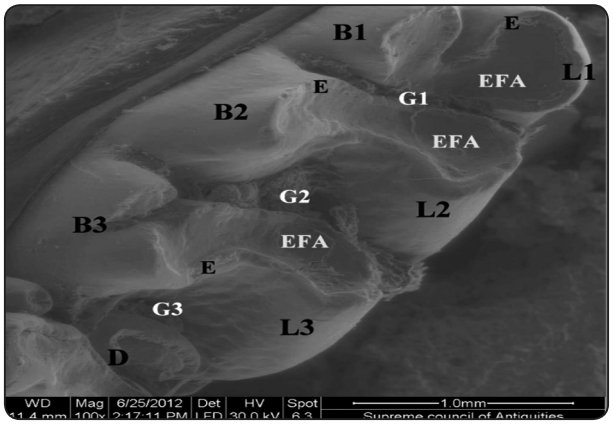

Fig. (1): SEM image (100x) of control group showing number and distribution of cusps and grooves of mandibular first molar of rat.

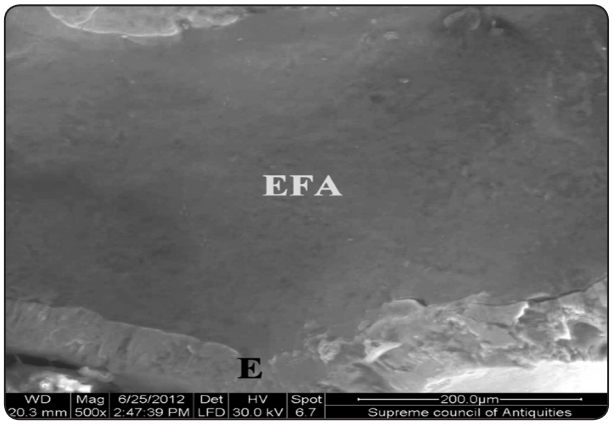

Fig. (2): SEM image (500x) of control group showing the occlusal aspect of each cusp which is covered by enamel at its margins (E) with the remaining area of enamel free area (EFA).

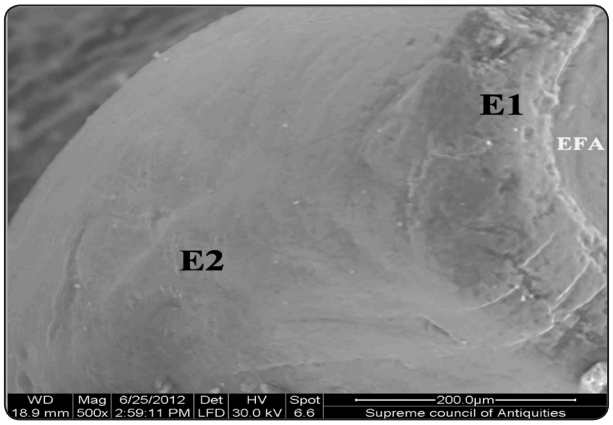

Fig. (3): SEM image (500x) of control group showing smooth enamel surface of buccal aspect (E2).

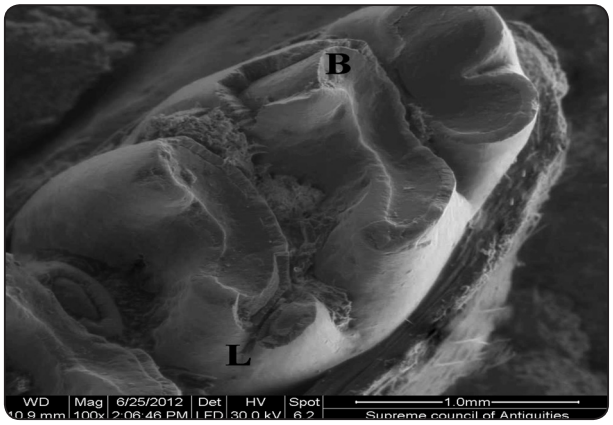

Fig. (4): SEM image (100x) of diabetic group showing severe erosion and defective enamel surface of buccal, lingual and distal cusps.

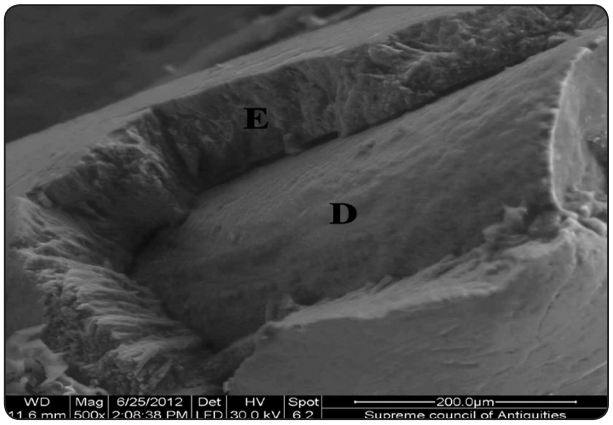

Fig. (5): SEM image (500x) of Buccal cusp of diabetic group showing exposure of the underlying dentin due to marked erosion of enamel.

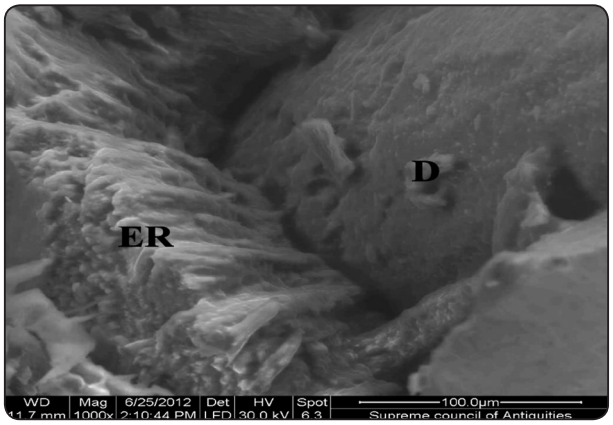

Fig. (6): SEM image (1000x) of diabetic group showing erosion of enamel rod (ER) by higher magnification.

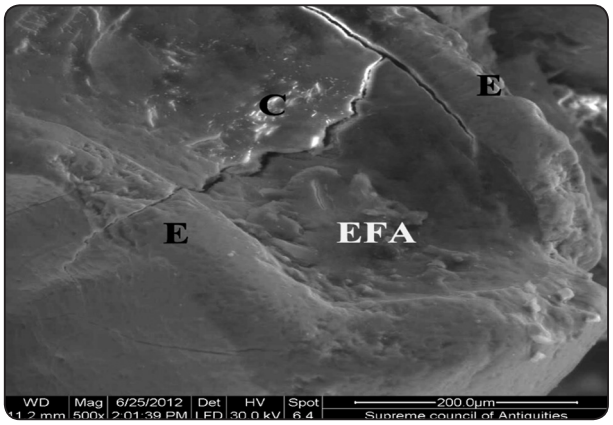

Fig. (7): SEM image (500x) of diabetic group showing mild cracking (C) along the occlusal surface within enamel as well as enamel free areas.

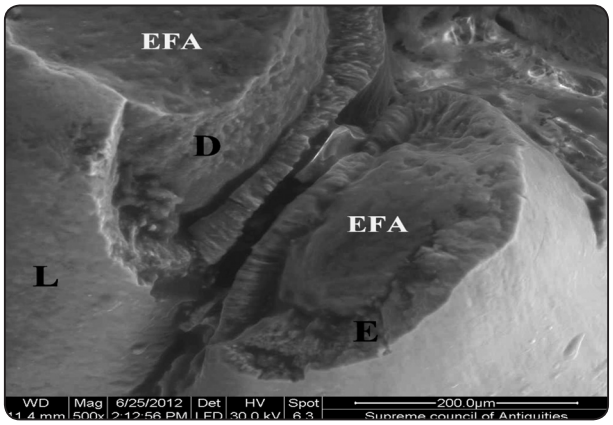

Fig. (8): SEM image (500x) of diabetic group showing intense defects as deep cracking along the occlusal surface within the enamel as well as enamel free areas and extending into the lingual surface.

Elemental Analysis Results using Electron Dispersive Analytical X-ray (EDAX):

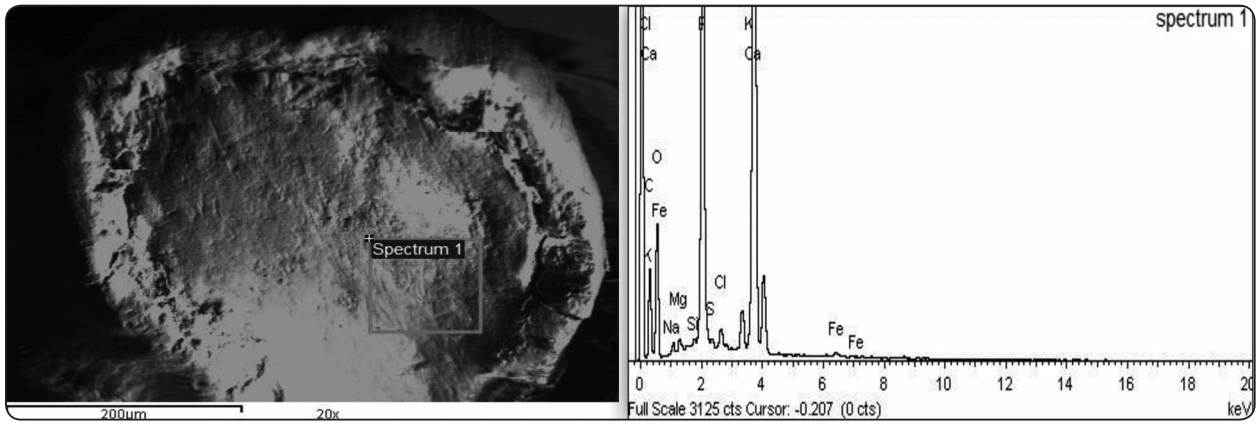

Fig. (9) SEM image (500) and EDAX surface high levels of Ca and P in control group.

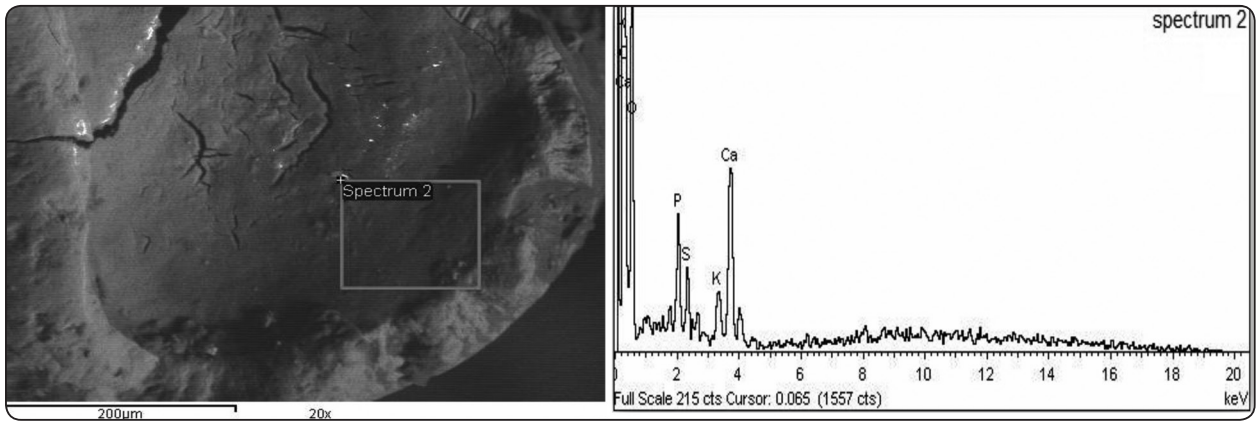

Fig. (10): SEM image (500) and EDAX surface low levels of Ca and P in diabetic group.

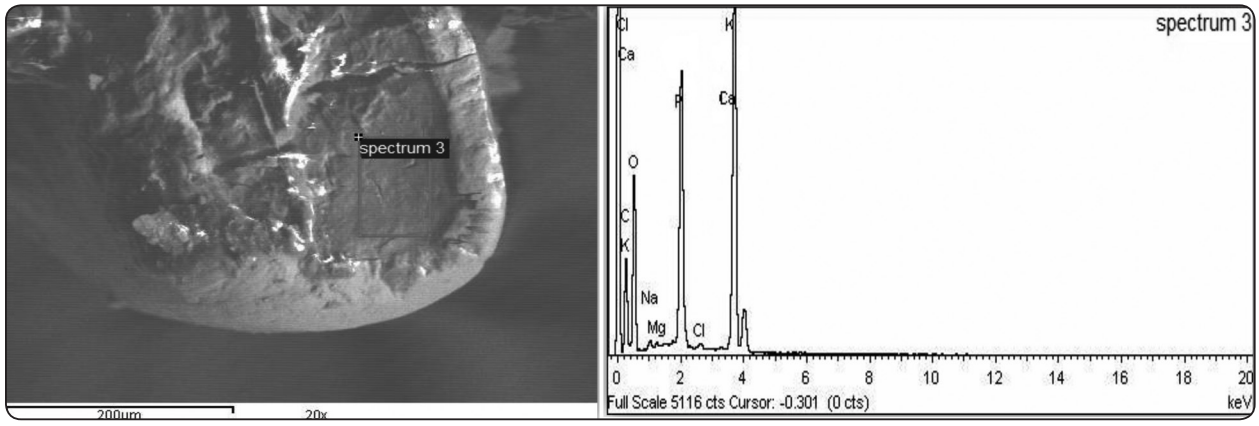

Fig. (11) SEM image (500) and EDAX surface low levels of Ca and P in diabetic group.

DISCUSSION

Maternal diabetes mellitus has long been associated with occurrence of birth disorders in both human[8,13] and experimental animals [7,14]. Nancy[15] reported that, the development of the dentition could be affected by several factor, including disturbance of calcium and phosphate, vitamins and metabolic disorders, endocrinopathies (including diabetes mellitus), inflammation and post radiation injuries. These factors don't act only during pregnancy (in utero) but also can act during neonatal and postnatal period. So the goal of the current study was to investigate the enamel surface alterations in molars of young rats of diabetic mothers. Diabetes may be inducted surgically by pancreatectomy[16], or chemically by streptozotocin [17] or alloxan [18]. We selected the chemical induction by administration of alloxan as it is simple to be carried out, producing a high proportion of diabetic rats and has been vastly utilized at our institution. It was efficient in production of hyperglycemia in about 78.8% of rats treated with alloxan. we made induction of diabetes before intermarriage to avoid the damaging acute poisonous impacts of alloxan on the embryos [19]. In our experiment, all off springs were sacrificed at 6 weeks after birth to ensure complete eruption of molars as the molars erupt on the 19th (1st molar), 22nd (2nd molar) and 35th to 40th (3rd molar) day. By 6 weeks the entire set is in use[20].

In the present study, ultrastructural results of control group showed that, enamel surface was smooth and homogenous while the diabetic group revealed defective enamel with rough surface. In some areas erosion and cracking were mild while in other areas were more intense leading to total loss of enamel and exposure of the underlying dentin. Loss of enamel in some areas was indicative of disturbed organic matrix formation, decreasing the enamel thickness. These results can be explained by Shaffer et al.,[21]who reported that a prolonged disorder in ameloblasts function might leads to absence of enamel in some areas. The rate at which the enamel hypoplasia occurs was extremely dependent upon the seriousness of hyperglycemia in the mother.

Complications of diabetes are not only dependent on hyperglycemia but also to multiple intracellular metabolic disorders associated with deficiency of glucose in a great number of cells. Interference with other biological pathways or induction of morphological alteration in distant areas might occur as a result of metabolites produced in these disturbed processes [22]. Additionally , Magnesium disorders might represent an another explanation to our results, due to that hypomagnesemia has been associated with diabetic animals [23], diabetic women and their neonates [24].

Our results were also in agreement with Adler et al., [25] and Grahnén et al., [26] who reported that, enamel hypoplasia and defects are more possible to be present in primary dentition of offspring of diabetic mothers. Similarly, Ghanim et al.,[27] reported that, enamel hypoplasia is a main disorder commonly happen in deciduous dentition in offspring of diabetic mothers. Disturbance of enamel matrix secretion may play an important role in producing defective enamel of tooth crown surface. It is a deterioration in the quantity of enamel resulting in its thickness reduction. It may occurs on smaller or bigger areas of the enamel in form of holes, grooves, partial or its total loss .

In another study, Karim [28] determined alloxan's influence on amelobalsts enamel secretion. He discovered some variations of the morphology in dentition that might be correlated to alloxan and hyperglycemia. Atar et al.,[29] have demonstrated the qualitative ultrastructural differences of enamel between diabetic and control rodents, suggesting that diabetes mellitus has a destructive influence on the developing enamel ultrastructure .

Additionally, both in diabetic patients and in experimentally induced diabetes in animals, exposure of the tissues to the activity of glucose due to chronic hyperglycemia leads to its damage and deterioration [30,31]. The surplus of output of conversion of glucose and synchronous ROS results in enzymes suppression which represents an

important cause of alteration in the constitutions and works of the body 's organs in diabetic persons [31,32]. Depending on clinical and experimental studies, it was concluded that, in diabetes disruption of ion transport might be occur as a results of oxidative stress [33]. Important factors in the pathogenesis of diabetes are inflammation and oxidative stress and they both significantly participate in the pathogenesis of diabetic complications[34].

Two mechanisms are included in the pathogenesis of diabetic adverse side effects. First, the polyol pathway through which glucose was converted into the enzyme sorbitol by aldose reductase that leads to tissue injury and other multiple diabetic side effects. Second, binding of glucose to proteins, lipids and nucleic acids which are responsible for formation of advanced glycosylation end products (AGE), the latter results in numerous changes in structures and functions, in addition to its precipitation in certain organs that leads to different side effects [35,36] . Similarly, Taylor and Borgnakke, [37] reported that, advanced glycation end products (AGEs) formation is considered to be major causal factors that contribute to the pathogenesis of diabetes and its side effects.

On the other hand, Zaw and Stone [38] reported that, numerous potential teratogens as hyperglycemia, hypoxia, ketone and amino acid abnormalities, and glycosylation of proteins may change the molecular signaling pathways and negatively influence the embryogenesis. Hyperglycemia might be contribute to liberation of free radicals leading to the disturbance in signal transduction. In diabetic patients, accumulation of serum free fatty acids and branched chain amino acids are considered to be a leading factors in diabetic complications.

Concerning the mineral analysis by energy dispersive x-ray (EDAX) which was carried out in our study to distinguish any alterations in minerals proportion in enamel of non-diabetic control and young rats of diabetic mother. The mechanism of EDAX analysis is dependent on bombing the specimens with high voltage electrons beams. The energy levels of refraction of that beams are different according to different types of minerals. Alteration in the energy levels reflects the alteration in mineral ratio. EDAX technique analysis is characterized by being accurate and non-invasive. Our work revealed that there was a decrease in the summit profiles of calcium and phosphorous of diabetic group compared with the control group, altering the calcium to phosphorus ratio. This is supported by previous studies using EDAX revealed a decrease in the content of calcium and phosphorus in all teeth in diabetic mice and rats, with phosphorus being more decreased than calcium [29]. This current study would appear to suggest that enamel malformation in the diabetic animals might be due to decreased secretional and maturational function of the ameloblasts, rather than through a deficiency of calcium and phosphorus.

Numerous reports have reported that, calcium plays a remarkable role in the formation of hydroxyapatite crystals which are essential for enamel remineralization[39]. In all probability, reduced cellular activity of ameloblasts inducing reduction of calcium incorporation into the enamel that might result in flaws of the tooth in diabetic cases [29].

Confirming our results, disturbed intracellular calcium metabolism might constitute a widely spread, underlying aberration that connecting the metabolic, cardiovascular, ocular and neural effects of diabetes [40]. Accumulative proof shows that impairment of Ca^{2+} homeostasis is a characteristic features of diabetes leading to its side effects . Disorders in cellular calcium regulation were present in erythrocytes, platelets, kidney, aorta, adipocytes, liver, osteoblasts, arteries, lens, peripheral nerves, retinal tissue, pancreatic beta cells, cardiac and skeletal muscles supporting that this disturbance in cellular calcium metabolism is a basic pathology linked to diabetes [41].

Additionally, other studies revealed that, a paradoxical metabolic imbalance in phosphate (P) happens from the early onset of diabetes and

might reduce the high energy phosphates and tissue hypoxia[42]. Diabetic patients manifested a lower levels of serum phosphorus as compared with healthy subjects; this may indicate a possible negative effect of hyperglycemia on serum phosphorus. Also Low phosphate levels were known to affect diabetic patients because diabetes has been reported to influence the phosphorus excretion by renal tubules[43,44].

CONCLUSION

The present study demonstrated that, alloxan induced diabetes had deleterious enamel surface alterations on molars of rats born to diabetic mothers. A reduction in the content of calcium and phosphorous between the control and the diabetic group, altering the calcium to phosphorus ratio. Therefore, diabetic patients' dental problems should be handled carefully and their diabetic condition monitoring is of prime importance, especially during early stage of tooth development. Furthermore, additional experiments are required to investigate the effects of maternal diabetes on other dental tissues.

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