AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Ultrastructural Study of the Effect of Omega-3 on Developing Rat Molars

[1]Azza Koura and [2]Nancy Saad

[1]*Associate Professor at Oral Biology Department, Faculty of Dentistry, Alexandria University, Egypt.*
[2]*Lecturer at Oral Biology Department, Faculty of Dentistry, Alexandria University, Egypt.*

Address For Correspondence:
Azza Koura, Associate Professor at Oral Biology Department, Faculty of Dentistry, Alexandria University, Egypt.

ARTICLE INFO

Article history:
Received 28 May 2016
Accepted 29 July 2016
Published 17 August 2016

Keywords:
Diabetes, Streptozotocin, Omega3,TransmitionElectron Microscope, Developing rat molars.

ABSTRACT

This current study is done to assess the effect of omega 3 on the developing rat molars by using transmition electron microscope (TEM). In this study fifteen pregnant healthy albino rats obtained from the Institute of Medical Research, Alexandria University were used. They were divided into three equal groups. Group (1) fed basal diet; group (II) fed basal diet and was induced diabetes by a single intravenous injection of Streptozotocin 60 mg/kg body weight in 0.1 M citrate buffer. Group (III) was induced diabetes and fed on basal diet modified with flaxseed. After delivery, the offsprings were sacrificed at day one and their developing mandibular first molars were examined by transmition electron microscope. The results revealed that developing molars of group (II) showed damaged ameloblast and odontoblast cells regarding the neuclus, cell organelles and cell junction. While those of group (III) showed marked improvement of the cell organelles and cell junction.

INTRODUCTION

Metabolic disorders recognized by the World Health Organization (WHO) included diseases whose prevalence is reported to be more increasing in the developing countries. The beneficial effects of omega-3 fatty acid consumption during diabetes have been shown from experimental and clinical studies. (Mori TA *et al*., 1999) and (Yilmaz O *et al*., 2002). Omega-3 polyunsaturated fatty acids (PUFAs) are increasingly being used as a nutritional strategy to prevent cardiovascular diseases, diabetes and obesity. Although the mechanism of action of omega-3 fatty acids remains unclear, the lipid-lowering action may be the cause of its beneficial effects on diabetes as suggested by different studies. (Yilmaz O *et al*., 2002)

Regarding the existence of favorable effects of omega-3 (PUFA) in normal pregnancy or in the treatment and prevention of diabetes during pregnancy and its outcomes on the offspring still an issue of debate. The use of omega- 3 (PUFA) supplementation has been recommended by previous studies, especially during pregnancy and lactation to reduce the risk of having preterm birth.

Some studies reported its beneficial effects on fetal development, visual and cognitive development. (Helland IB *et al*., 2006; Koletzko B *et al*., 2007) While other authors have found that omega -3 (DHA) Docosahexaenoic acid supplementation (800mg/day) during the second half of human pregnancy does not reduce the risk of gestational diabetes mellitus(GDM) or preeclampsia in mothers, others have shown that (DHA) supplementation can reduce the risk of perinatal death and neonatal convulsions in newborns.(Zhou SJ *et al*., 2012)

Previous epidemiological and clinical trials that have shown that preexisting maternal diabetes (type 1 and type 2) or GDM appears to be an important risk factor for fetal over nutrition and macrosomia.(Evers IM *et al*., 2004; Dörner G *et al*., 1987) Guidelines from the Polish Gynecological Association recommended the use of omega-3 PUFA either as supplements or through dietary counseling for women who are planning pregnancy and

To Cite This Article: Azza Koura and Nancy Saad., Ultrastructural Study of the Effect of Omega-3 on Developing Rat Molars. ***Aust. J. Basic & Appl. Sci.,*** *10(12): 275-283, 2016*

for patients with normal and/or gestational diabetes and during lactation, cited by.(Page and Eke, 2007; Moher D *et al*., 2012) Moreover, the American Pregnancy Association reports the recommendation of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) which stated that pregnant women should take 300mg minimum to support themselves and the fetus with DHA requirements on a daily basis.(American Pregnancy Association, 2014; The Omega-3 FAQ)

The oral tissue structure, Like other tissues of the body is also affected by birth prematurity or as a response of the oral tissues to medical problems of preterm children during the neonatal period.(Seow WK *et al*., 1989)

Changes in enamel are one of the most noticeable oral effects of preterm birth, and may classically present as enamel hypoplasia which is defined as a quantitative loss of enamel, or as enamel opacity which is defined as a qualitative change in the translucency of the enamel.(Seow WK, 1997)

Dental enamel is the most highly mineralized and hardest tissue covering the crowns of vertebrate teeth.(Setchell ED *et al*., 2003) It has long been recognized that continuously erupting rodent incisors have considerable potential to serve as a model system for amelogenesis.(Duthie GG *et al*., 1998; Young JF *et al*., 1999)

Dentinogensis takes place in two phases. First is the organic matrix formation, followed by the deposition of calcium phosphate (hydroxyapatite) crystals in the matrix. A well-developed system of microtubules is present within both the cell body and odontoblastic process. They have important functions in the transport of intracellular structures, especially secretion of granules. Also, they play a role as cytoskeletal element to stabilize cellular shape and cell surface components.(González-Periz A *et al*., 2009)

The features of the intra – odontoblastic collagen fibrils of the mouse were found most common in secreting odontoblasts of the maturing stage, mostly at the peripheral zone of the Golgi apparatus, and were sometimes seen in odontoblastic processes. Intra-odontoblastic collagen fibrils had morphological variation including a banded structure enclosed by limiting membranes of vacuoles, they might show fusion with primary lysosomes, or appeared as an electron-dense material covered with a structure that was not banded. (Arts and Hollman, 2005)

The assembly of the collagenous organic matrix prior to mineralization was studied ultrastructurally in predentin of rat incisors, they observed that, progressing from the odontoblastic surface to the mineralization front, the collagen fibrils thicken to form a dense network, and their banding pattern varies. (D`Archivio M *et al*., 2007)

Several modes exist for inducing experimental maternal diabetes with streptozotocin in animal models and the consequences on fetus and adult progeny are variable with each model.(Van Assche FA *et al*., 2001; Aerts L *et al*., 1990) The streptozotocin, when administered at a high single dose, induces diabetes by the direct toxic effects on pancreatic β-islet cells.(Van Assche FA *et al*., 2001) The fetus is confronted with severe intrauterine hyperglycemia which induces fetal islet hypertrophy and β-cell hyperactivity and may result in early hyperinsulinemia.(Aerts L *et al*., 1990) The increased insulin secretion dramatically and rapidly decreases due to the overstimulation of fetal β cells which are depleted of insulin granules, resulting in fetal hypoinsulinemia.(Van Assche FA *et al*., 2001; Aerts L *et al*., 1990)

MATERIALS AND METHODS

Total number of nine males & fifteen females' healthy albino rats (obtained from the Institute of Medical Research, Alexandria University, Egypt) with average weight of 225±25 grams were used. When the females were recognized to be pregnant by palpation and by sharp weight gain, they were separated into individual cages.

A basal diet (BD) was formulated to cover all the essential nutrients for pregnant rats according to AIN-93G diet in order to meet the nutritional needs of developing and lactating rats.(Reeves PG *et al*., 1993) A flaxseed diet (FSD) was prepared by supplementing the basal diet with 10% ground flaxseed after adjustment for total calories, macronutrients, and fiber contributed by the added flaxseed, as described.(Chen J *et al*., 2003) Fresh diet was stored at 4°C and its intake was monitored throughout the study. The experimental period was extended for twenty one days. The day of birth was designed as day one.

The animals were divided into three groups as follows:

Group (1): five mothers fed basal diet (control group).

Group (II): five mothers with induced diabetes and fed with basal diet (diabetic, BD).

Group (III): five mothers with induced diabetes and fed with basal diet modified with flaxseeds (diabetic, FSD).

For group (I) the rats were injected with vehicle (citrate buffer) to simulate the influence of injection stress or buffer-induced effects on the animals.(Tierney LM *et al*., 2002)

Induction of diabetes: For groups (II) and (III), the rats were fasted overnight and diabetes were induced by a single intravenous injection of Streptozotocin 60 mg/kg body weight in 0.1 M citrate buffer.(World Health Organization, 1999)

Australian Journal of Basic and Applied Sciences, 10(12) July 2016, Pages: 275-283

Total number 15 Offsprings, were sacrificed from three groups (five from each) at day one after delivery. Animal sacrification: At the day of sacrification each animal was anesthetized, then sacrificed with cervical dislocation. The head was separated from the body, and fixed in 10% neutral buffered formalin for 3 days. The dissected mandibles were prepared to examine the area of the first molar by transmition electron microscopic examination (TEM).

Results:

Developing rat mandibular 1st molar (control group), showed odontoblast cells with normal appearance of desmosomal junction and prominent tonofilaments between the adjacent cells. Mitochondria with lamellated cristae. Free ribosomes and polysomes also appeared (fig 1).

In diabetic group with basal diet (Diabetic, BD):- odontoblast cells of the developing rat mandibular first molar were poorly differentiated with abnormally wide intercellular spaces. Cytoplasm showed free ribosomes as well as shrunken mitochondria with electron dense matrix and ill defined cristae. Nuclei appeared of different shape and size. The distal cytoplasm of odontoblast cells revealed condensation of vacuoles containing procollagen. Large amount of the ribosomes and polysomes were filling the cytoplasm (fig 2, 3, 4). Ameloblast cells showed few altered mitochondria with electron dense matrix. The intercellular spaces appeared abnormally wide. Reduced number of desmosomal junction (fig 5).

In diabetic group with flaxseed enriched diet (diabetic, FSD):- Odontoblast cells of the developing first molar showed euchromatic nucleus, regular nuclear margin and multinuceoli were evident. Cytoplasm revealed great amount of ribosomes, polysomes and profiles of moderately dilated rER cisternae, which were showing well organized tall columnar cells with elongated nuclie arranged toward the basal end of the cells. Euochromatin predominate and heterochromatin is restricted on the nuclear margin. Multiple nucleoli were evident where the nuclei occupy most of cell width leaving narrow band of cytoplasm between it and lateral cell membrane. The lateral cell membranes are close and straight. Mitochondria with electron dense matrix and less defined cristae were also found (fig 6,7). Secretory ameloblast cells of the mandibular first molar appeared well organized tall columnar cells with elongated nuclei arranged toward the basal end of the cells. Euochromatin predominated and heterochromatin was only seen on the nuclear margin. Multiple nucleoli were also evident. The nuclei occupied most of cell width; a narrow cytoplasm band appeared between the nucleus and lateral cell membrane, which were properly close and regular. (Fig 8,9).

Control group (group I):

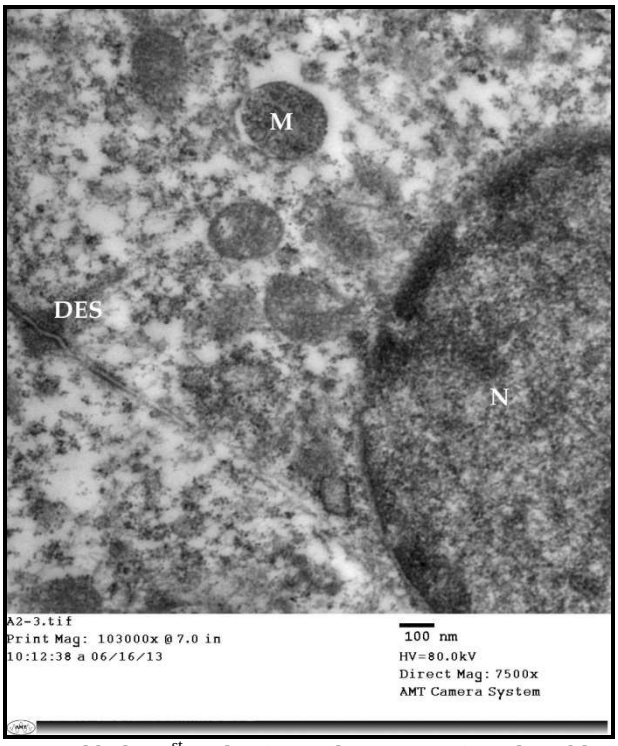

Fig. 1: E.M in developing rat mandibular 1st molar (control group, BD) :-odontoblast cells showing desmosomal junction (DES) with prominent tonofilaments between the adjacent cells. Euochromatic Nucleus appears

with smooth nuclear membrane (N). Mitochondria (M) with lamellated cristae .Free ribosomes and polysomes are also observed. (X7500).

Diabetic with BD group (II):

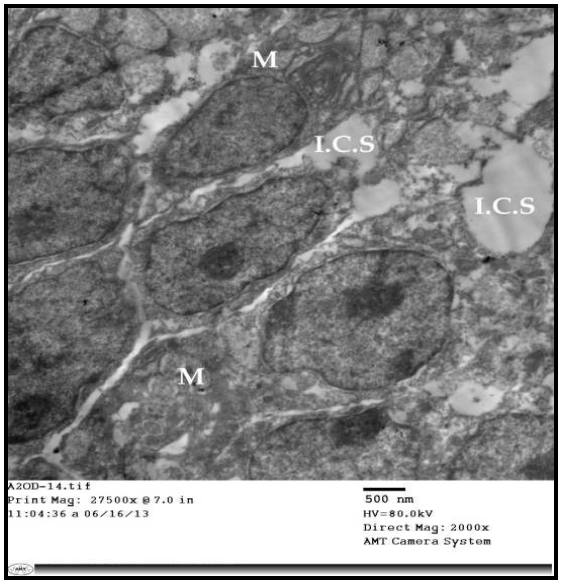

Fig. 2: E.M of odontoblasts in developing rat mandibular first molar (diabetic group, BD) group (II) showing, poorly differentiated cells with abnormally wide intercellular spaces (I.C.S). Cytoplasm showing free ribosomes & shrunken mitochondria (M) with electron dense matrix (X2000)

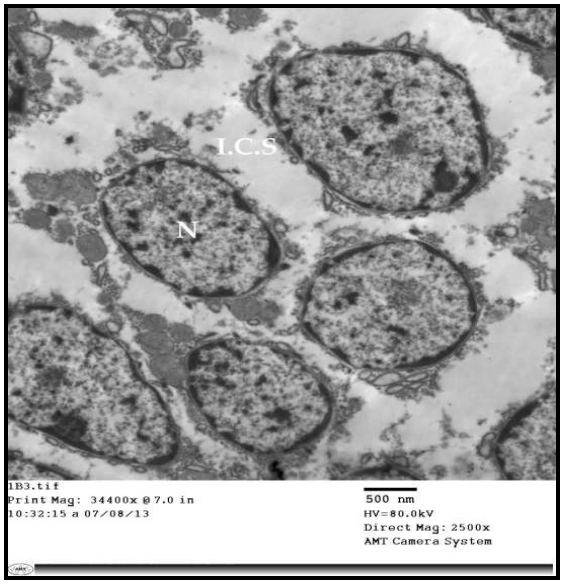

Fig. 3: E.M of the developing rat mandibular 1st molar (diabetic group, BD) group (II) showing ameloblast cells with severe widening of the intercellular spaces (ICS). Nuclei (N) appear of different shape and size. The mitochondria are evident with ill defined cristae and electron dense matrix (X2500).

Australian Journal of Basic and Applied Sciences, 10(12) July 2016, Pages: 275-283

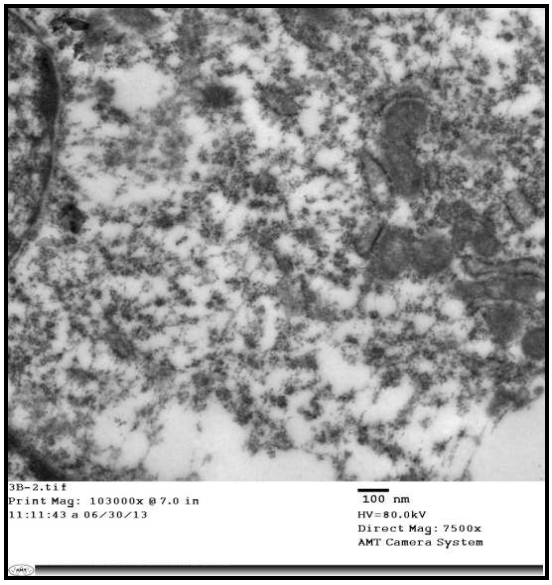

Fig. 4: E.M of the developing rat mandibular 1st molar (diabetic group, BD) group (II) showing the distal cytoplasm of odontoblast cells with condensation of vacuoles containing procollagen. Large amount of the ribosomes and polysomes filling the cytoplasm are seen. (X7500).

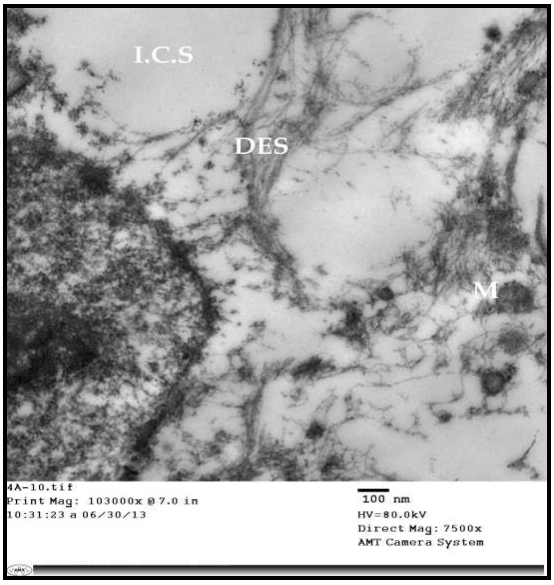

Fig. 5: E.M of ameloblast cell from rat mandibular 1st molar (diabetic group, BD) group (II) showing few altered mitochondria (M) with electron dense matrix. The intercellular spaces (ICS) appear abnormally wide. The desmosomal junction (DES) reduced in number (X7500).

Diabetic with (FSD) group:-group (III):

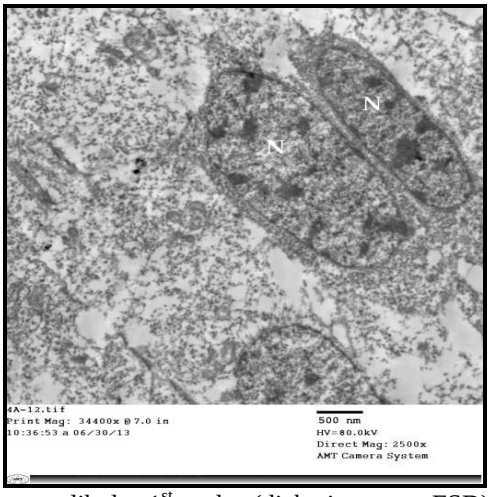

Fig. 6: E.M of the developing rat mandibular 1st molar (diabetic group, FSD) group (III) showing odontoblast cells with euchromatic nucleus (N), regular nuclear margin and multinuceoli can be seen. Cytoplasm with great amount of ribosomes and polysomes were also detected (X2500).

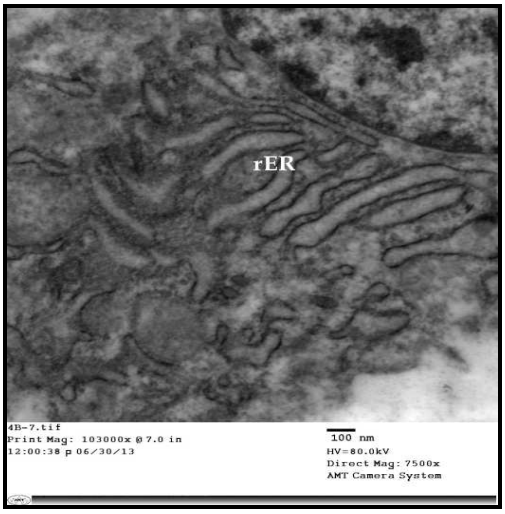

Fig. 7: E.M in supranuclear cytoplasm of secretary odontoblast of developing rat mandibular 1st molar (diabetic group, FSD) group (III) showing profiles of rER cisternae, which are mildly dilated. Mitochondria with electron dense matrix and less defined cristae are observed (X7500).

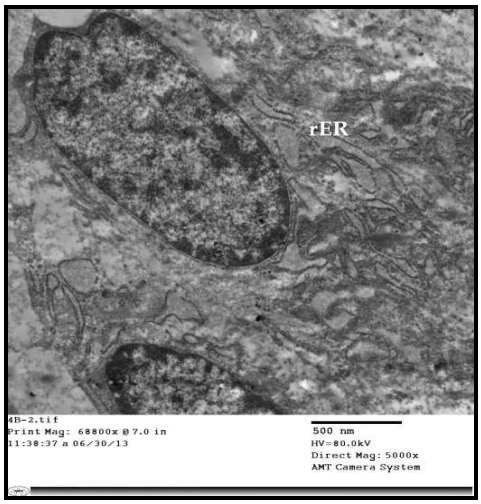

Fig. 8: E.M of ameloblast cells of developing rat mandibular 1st molar (diabetic group, FSD) group (III), showing euochromatin predominates in the nucleus and heterochromatin is only seen adjacent to the nuclear margin. Moderately dilated rER cisternae are seen in the distal cytoplasm. Condensation vacuoles containing procollagen are evident. (X5000).

Australian Journal of Basic and Applied Sciences, 10(12) July 2016, Pages: 275-283

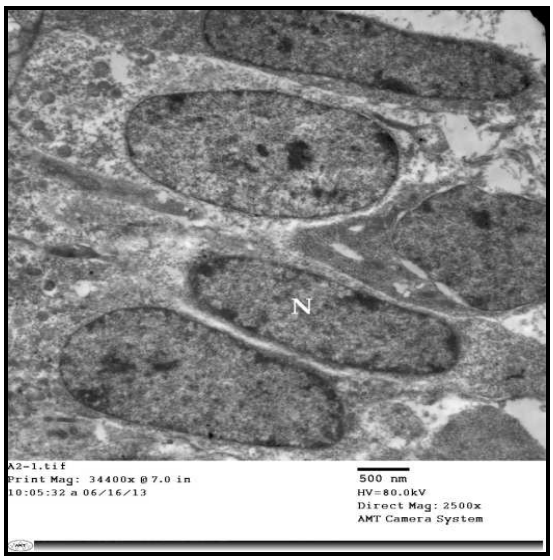

Fig. 9: E.M of secretory ameloblast cells from developing rat mandibular first molar (diabetic, FSD) group (III) showing well organized tall columnar cells with elongated nuclei (N) arranged toward the basal end of the cells. Euochromatin predominate and heterochromatin is restricted on the nuclear margin.The nuclei occupy most of cell width leaving narrow band of cytoplasm between it and lateral cell membrane. Multiple nucleoli are evident.The lateral cell membranes are close and straight (X2500)

Discussion:

Diabetes is one of the most frequent diseases that could affect a large number of populations. Its hyperglycemic state is associated with a large number of complications, leading to retinopathy, neuropathy, nephropathy, atherosclerosis, periodontitis and impaired wound healing.(Seppela B *et al*., 1993)

Streptozotocin (STZ) was used in this study as diabetes inducer, as it had been used by many researchers around the world to create experimental diabetes for its simple effective and available method.(Graves DT *et al*., 2006) In the current study the widening of the intercellular spaces which were observed in group(II) might reflect a delay in the differentiation of the cells or might be due to alteration with the normal organization of the cells. This could interphere with normal exchange of nutrients. The presence of degenerated nuclei, chromatin condensation which is found in group (II) may be a result of release of apoptotic factors from degenerated mitochondria. These phenomena include mitochondrial alterations e.g. production of reactive oxygen species, i.e., nitroxidative stress by nitric oxide or similar compounds and mitochondrial membrane permeabilization, lysosomal changes, increases in the cytosolic concentration of calcium (ca2+) that result in mitochondrial overload. This is in accordance with Devasagayam (2004). (Devasagayam TP *et al*., 2004) The predominance of heterochromatin which is inactive in protein synthesis over the finely dispersed euchromatin in the nuclei of some cells, indicate that the metabolic activity of the cells was impaired, and these cells became inactive. Same results were also obtained by Rhodin (1983).(Rhodin and Miyata, 1983) The impaired structure of the ameloblast and odontoblast cells is in accordance with Chin-ko *et al* 2012 who stated that hyperglycemia adversely affects enamel matrix protein and pulp repair.(Yeh CK *et al*., 2012) As well as the energy dispersive Xray (EDX) analysis revealed a reduction in the amount of calcium and phosphorus in all regions affected by diabetes. In the same time it was stated that type 2 diabetes (in pregnant mothers) can affect the normal tooth development in human, M.Atar 2004.(Atar M *et al*., 2004) High blood glucose has been shown to induce an oxidative stress which in turn induces the production of highly reactive oxygen species toxic to cells particularly the plasma membranes where the radicals interact with the lipid bilayer.(Ceriello and Motz, 2004) On the other hand the presence of large quantities of ribosomes and polysomes in group (III) are linked to the cells undergoing rapid growth. This is in accordance with the results found by Devasagayam *et al* (2004). (Devasagayam TP *et al*., 2004) Well improvement was observed in the ameloblast and odontoblast cells of group(III) at the ultrastructural level, indicating almost normal functioning cells. Some studies have reported that dietary supplements with vitamins and minerals prevent or at least attenuate the organic deterioration caused by an excessive oxidative stress associated with diabetes in humans and animals.(Kamath U *et al*., 1998) This argument is supported by the findings that dietary fish oil modulates the composition of plasma membrane phospholipids by increasing omega-3 PUFA contents (EPA and DHA in particular) at the expense of arachidonic acid (AA, an omega-6 PUFA) levels.(Ylönen K *et al*., 2003)

Conclusion:

Australian Journal of Basic and Applied Sciences, 10(12) July 2016, Pages: 275-283

From the present study it is concluded that a moderate level of omega-3 PUFA dietary intake could be beneficial for improving the histological structure of the ameloblast and odontoblast cells and subsequently matrix formation of enamel and dentin of developing rat molars which are damaged by being delivered from diabetic mothers. PUFA intake is recommended for pregnant mothers especially those with DM to prevent or decrease the damaging effect of the disease on the developing teeth of human embryos and this might need further researches.

REFERENCES

Aerts, L., K. Holemans and F.A. Van Assche, 1990. Impaired insulin response and action in offspring of severely diabetes rats. In: Shafrir E (ed). Frontiers in Diabetes Research. Lessons from Animal Diabetes III. London, UK: Smith-Gordon, pp: 561-6.

American Pregnancy Association, 2014. Omega-3 Fish Oil and Pregnancy: Dosage Recommendations. available at: http://www.American pregnancy.org/pregnancy-health/omega-3-fish-oil/.

Arts, I.C.W and P.C.H. Hollman, 2005. Poyphenols and disease risk in epidemiologic studies. Am J Clin Nutr, 81: 317-25.

Atar, M., D.R. Atar-Zwillenberg, P. Verry and U.M. Spornitz, 2004. Defective ultastructure in diabetic rodents. Int J Paediatr Dent, 14: 301-7.

Ceriello, A and E, Motz., 2004. Arterioscelerosis, Thrombosis, and Vascular Biology. Am Heart Assoc, 24: 816-23.

Chen, J., K.P. Tan, W.E. Ward and L.U. Thompson, 2003. Exposure to flaxseed or its purified lignan during suckling inhibits chemically induced rat mammary tumorigenesis. Exp Biol Med (Maywood), 228: 951-8.

D`Archivio, M., C. Filesi, R.D. Benedatto, R. Gargiulo, C. Giovannini and R. Masella, 2007. Polyphenols dietary sources and bioavailability. Ann ISE Super Solna, 43: 348-61.

Devasagayam, T.P., J.C. Tilak, K.K. Boloor, K.S. Sane, S.S. Ghaskadbi and R.D. Lele, 2004. Free radicals and antioxidants in human health: Current Status and Future Prospects. J Assoc Physicians India, 52: 794-804.

Dörner, G., A. Plagemann and H. Reinagel, 1987. Familial diabetes aggregation in type I diabetics: gestational diabetes an apparent risk factor for increased diabetes susceptibility in the offspring. Exp Clin Endocrinol, 89: 84-90.

Duthie, G.G., M.W. Pedersen, P.T. Gardner, P.C. Morric, A.M. Jcnkinson, D.B. McPhail and G.M. Steele, 1998. The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. Eur J Clin Nutr, 52: 733-6.

Evers, I.M., H.W. de Valk and G.H. Visser, 2004. Risk of complications of pregnancy in women with type 1 diabetes: nationwide prospective study in the Netherlands. BMJ, 328: 915.

González-Periz, A., R. Horrillo, N. Ferre, K. Gronert, B. Dong, E. Moran-Salvador, *et al*., 2009. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J, 23: 1946-57.

Graves, D.T., R. Liu, M. Alikhani, H. Al-Mashat and P.C. Trackman, 2006. Diabetes enhanced inflammation and apoptosis-impact on periodontal pathology. JDR, 85: 15-21.

Helland, I.B., O.D. Saugstad, K. Saarem, A.C. Van Houwelingen, G. Nylander and C.A. Drevon, 2006. Supplementation of n- 3 fatty acids during pregnancy and lactation reduces maternal plasma lipid levels and provides DHA to the infants. J Matern Fetal Neonatal Med, 19: 397-406.

Kamath, U., G. Rao, C. Raghothama, L. Rai and P. Rao, 1998. Erythrocyte indicators of oxidative stress in gestational diabetes. Acta Paediatr, 87: 676-9.

Koletzko, B., E. Larqué and H. Demmelmair, 2007. Placental transferof long-chain polyunsaturated fatty acids (LC-PUFA). J Perinat Med, 35(Suppl 1): S5-11.

Moher, D., S. Hopewell, K.F. Schulz, V. Montori, P.C. Gotzsche, P.J. De¬vereaux, *et al*., 2012. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. Int J Surg, 10: 28-55.

Mori, T.A., D.W. Dunstan, V. Burke, K.D. Croft, J.H. Rivera, L.J. Beilin, *et al*., 1999. Effect of dietary fish and exercise training on urinary F2-isoprostane excretion in non-insulin-dependent diabetic patients. Metabolism, 48: 1402-8.

Page, R.C and P.I. Eke, 2007. Case definitions for use in population-based sur¬veillance of periodontitis. J Periodontol, 78(7 Suppl): 1387-99.

Reeves, P.G., F.H. Nielsen and G.C. Fahey, 1993. AIN-93 purified diets laboratory rodents: final report of the American Institute of Nutrition adhoc writing committee on the reformulationfor of the AIN-76A rodent diet. J Nutr, 123: 1939-51.

Rhodin, A.G.J. and K. Miyata, 1983. Advances in Herpetology and Evolutionary Biology. Essays in Honor of Emest E. Willams. 13-32. Cambridge, Massachusetts: Mus Comp Zool.

Seow, W.K., J.P. Masel, C. Weir and D.I. Tudehope, 1989. Mineral deficiency in the pathogenesis of enamel hypoplasia in prematurely-born, very low birth weight children. Ped Dent J, 11: 297-302.

Seow, W.K., 1997. Effects of preterm birth on oral growth and development. Aust Dent J, 42: 85-91.

Seppela, B., M. Seppela and J. Ainamo, 1993. A longiotudinal study on insulin dependent diabetes mellitus and periodontal disease. J Clin Periodontal, 20: 161-5.

Setchell, E.D., M.S. Faughnan, T. Avades, L. ZimmerNechemias, N.M. Brown, B.E. Wolfe, *et al*., 2003. Comparing the pharmacokinetics of daidzein and genistcin with the use of 13C-labled tracers in premenopausal women. Am J Clin Nutr, 77: 411-9.

The Omega-3 FAQ. The definitive guide to omega 3 fatty acids: omega 3 recommendations and requirements: pregnant and nursing women. Available at: http://www.omega3faq.com/omega-3- dietary-recommendations/, http://dhaoil.org

Tierney, L.M., S.J. McPhee and M.A. Papadakis, 2002. Current Medical Diagnosis & Treatment. International edition. New York: Lange Medical Books\McGraw-Hill, pp: 1203-15.

Van Assche, F.A., K. Holemans and L. Aerts, 2001. Long-term consequences for offspring of diabetes during pregnancy. Br Med Bull, 60: 173-82.

World Health Organization., 1999. Department of Noncommunicable Disease Surveillance. Definition, Diagnosis and Calssification of Diabetes Mellitus and its Complications.

Yeh, C.K., S.E. Harris, S. Mohan, D. Horn, R. Fajardo, Y.H. Chun *et al*., 2012. Hyperglycemia and xerostomia are key determinants of teeth decay in type 1 diabetic mice. Lab Invest, 92: 868-82.

Yilmaz, O., Y. Ozkan, M. Yildirim, A.I. Oztürk and Y. Erşan, 2002. Effects of alpha lipoic acid, ascorbic acid-6-palmitate, and fish oil on the glutathione, malonaldehyde, and fatty acids levels in erythrocytes of streptozotocin induced diabetic male rats. J Cell Biochem, 86: 530-9.

Ylönen, K., G. Alfthan, L. Groop, C. Saloranta, A. Aro and S.M. Virtanesn, 2003. Dietary intakes and plasma concentrations of carotenoids and to copherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. Am J Clin Nutr, 77: 1434-41.

Young, J.F., S.E. Nielsen, J. Haraldsdottir, S.T. Daneshvar Lauridsen, P. Knuthscn, A. Crozier, *et al*., 1999. Effect of fruit juice intake on urinary queretin exceretin and biomarkers of antioxidative status. Am J Clin Nutr, 69: 87-94.

Zhou, S.J., L. Yelland, A.J. McPhee, J. Quinlivan, R.A. Gibson and M. Makrides, 2012. Fish-oil supplementation in pregnancy does not reduce the risk of gestational diabetes or preeclampsia. Am J Clin Nutr, 95: 1378-84.