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Nicotine exposure and its role in delaying the formation of long bones during embryonic development

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التعرض للنيكوتين ودوره في تأخير تكوين العظام الطويلة أثناء التطور الجنيني

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Abstract:
The purpose of this study is to figure out the effects of nicotine on embryonic development. Various abnormalities in skeletal bone formation and general morphology, were assessed using domestic chick Galls Galls embryos. The fertilized eggs were sterilized with 70% ethanol and then nicotine solutions at concentrations of 0.1, and 0.5 mg/ml were injected in the eggs on day zero before being placed in an incubator set at a temperature between 37.5 and 38 Cº. embryos were collected at specific developmental stage HH36 (Day 10). The examination of morphological changes in the embryos was conducted under a dissecting microscope, while defects in bone ossification were identified through the application of alizarin red stain. Consequently, this research will highlight that maternal exposure to nicotine during the early phases of embryonic development could potentially lead to a delay in bone ossification, resulting in reduced sizes of both long and flat bones.

Keywords: Nicotine Ossification, Skeleton, Embryonic Development.

الملخص
الغرض من هذه الدراسة هو معرفة آثار النيكوتين على التطور الجنيني المبكر. تم تقييم التشوهات المختلفة في تكوين العظام والهيكل العظمي والتشكل العام باستخدام أجنة الكتكوت المحلية Galls Galls. تم تعقيم البويضات المخصبة باستخدام 70٪ من الإيثانول ثم تم حقن محاليل النيكوتين بتركيزات 0.1 و0.3 و0.5 ملغم / مل في البيض في اليوم صفر قبل وضعها في الحاضنة عند درجة حرارة تتراوح بين 37.5 و38 درجة مئوية. وتم جمع الأجنة في اليوم العاشر من التحضين وهو ما يعادلHH36 . تم إجراء فحص التغيرات المورفولوجية في الأجنة تحت المجهر التشريحي، في حين تم تحديد العيوب في تعظم العظام من خلال تطبيق صبغة الالزارين الحمراء وتبين عدم اكتمال التعضم في لااجنه المتعرضه للنيكونين مقارنه بالاجنه التي لم تتعرض للنيكوتين، وبالتالي سيسلط هذا البحث الضوء على أن تعرض الأم للنيكوتين خلال المراحل المبكرة من التطور الجنيني يمكن أن يؤدي إلى تأخير في تعظم العظام، مما يؤدي إلى انخفاض أحجام العظام الطويلة والمسطحة.

الكلمات المفتاحية: النيكوتين، التعظم، التطور الجنيني.

Introduction

Congenital malformations, a structural condition resulting at birth, can be caused by genetic or environmental factors during the prenatal phase of development [1]. Teratogenic agents are more likely to cause significant congenital malformations during organogenesis [2]. Toxic elements from cigarettes enter the body through active smoking or passive smoking, which occurs when the body is exposed to a contaminated environment [3]. Smoking during conception can affect the development of crucial embryonic organs like the central nervous system (CNS), cardiovascular system (CVS), and skeletal system (SK). (Mone et al., 2004 Nicotine, a volatile alkaloid, is a colorless, water-soluble compound released during cigarette combustion, soluble in organic compounds and highly soluble in air and light [4].

Nicotine acts as a receptor agonist at most nicotine acetylcholine receptors (nAChRs), imitating acetylcholine, a naturally occurring substance in mammalian bodies [4]. Research indicates that nicotine's impact on stem cell

survival, apoptosis, proliferation, and differentiation depends on factors like cell types, nAChR subtypes, distribution, and binding density [5]. The effects of nicotine exposure, its concentration and frequency, and the functional status of nAChRs also impact these effects[6].

Further investigations have been conducted into the impact of nicotine on heart rate (HR). A study exploring the effects of nicotine on the cardiovascular system (CVS) in pregnant women revealed a correlation between nicotine and elevated maternal and fetal heart rates (HR)[7] . overview of current research on the subject. Your introduction should clearly identify the subject area of interest.

Bone, an ossified organ that constitutes a portion of the skeletal system, provides structural support to the body, safeguards the internal organs, generates red and white blood cells, stores minerals, and facilitates movement [8]. Diverse in shape and sizes, bones exhibit a sophisticated internal and external framework, despite their lightweight, durability, and rigidity. Comprised of vital cells embedded within a mineralized organic cellular matrix, bone consists of organic elements, predominantly collagen type I, alongside inorganic components, primarily hydroxylapatite and other calcium and phosphate salts [9]. The presence of nicotine in cigarettes hampers the production of osteoblasts[10]. Furthermore, smoking impedes the absorption of dietary calcium essential for bone development, resulting in weakened bones[11]. A study investigated the impact of nicotine on heart rate variability (HRV) in adolescents, aiming to discourage smoking and abuse. Results showed nicotine decreases heart rate in both male and female healthy individuals, with smoking particularly causing harm to the cardiac system. (Guo et al., 2022). exposure of a pregnant woman to secondhand smoke may result in mineralization complications in the skeletal structure of her offspring, thereby establishing a direct prenatal link to the heightened susceptibility to fractures noted in offspring of mothers who encountered cigarette smoke[12] nicotine exposure during pregnancy has been observed to impede the skeletal growth of developing fetuses [13]. The study aimed to figure out the effect of different nicotine solution concentrations on long bone in early embryonic development.

Material and methods

Nicotine concentrations were prepared in stock solution and used to clean chicken eggs for embryo position determination. Egg candling was performed, and nicotine concentrations were injected into control, distal water, and treated groups. Incubation took place at 37-38°C and 60-80% humidity. All embryos were staged according to Hamburger and Hamilton definitions and harvested at various stages.

Blunt forceps were used to scrap the eggshell over an embryo, creating a small window, cutting through the shell membrane, severing blood vessels, lifting embryos, transferring them to a petri dish, removing membranes, fixing them in a 10% formalin solution, and photographing the embryos on an agar plate using a digital camera**.**

The skeletal system of embryos was stained using a double staining method. The embryos were dehydrated in 70% ethyl alcohol, then in pure acetone, and then transferred to glass containers containing Alizarin Red stain. After 7 days, they were washed and treated with KOH for transparency. The embryos were preserved in different concentrations.

Results and discussion

5.3 Effect of nicotine on chicken embryo development at HH36

Figure (1) below shows an effect of nicotine on whole mount of chicken embryos injected at day zero and collected at stages HH36. It showed that control group, treated group injected with nicotine 0.1 Mg/ml and a treated group injected with nicotine 0.5 Mg/ml in stage HH36 were presented in (Fig 1 A and A') and (Fig 1 B and B') (Fig 1 C and C') consecutively. Control embryos without injected were collected at stage HH36, were also observed that both a control is growing normally, (Fig 1A) represents a control group and had no observed abnormalities, the embryo formation appears with the presence of the extremities, the distal parts of each wing and leg are relatively longer, the toes are flat laterally and curved ventrally, and the eyelids, the neck has grown normally. The length of the embryos in general 4.3 cm and the length of the control neck in general 1cm. (Fig 1 A') represents a control group, the hind limbs of the legs were growing normally and there was no deformity in them, as well as the length of the normally developing fingers and the length of the leg is normal and the distance between the joint and the leg is normal. Embryos were treated with 0.1 mg/ml group of nicotine (Fig 1 B), and the embryos showed slower growth compared to the control group, in addition to the deformation of the skull and that the legs of the embryos were abnormal, and when measuring the length of the neck, it was less than the control group, The length of the embryos in general 4 Cm and the length of the control neck in general 0.8Cm. (Fig 1 B') embryos showed abnormally when compared to control in the hind limbs of the leg the presence of toe deformity, as well as the length of the foot. The fingers grow abnormally, as well as the distance between the joint and the leg is short.

In study done by group of researcher in 2012 found that, nicotine leads to a decrease in exogenous estrogens through the inhibition of osteoblast activity, resulting in lower body weight and earlier onset of menopause [14] .

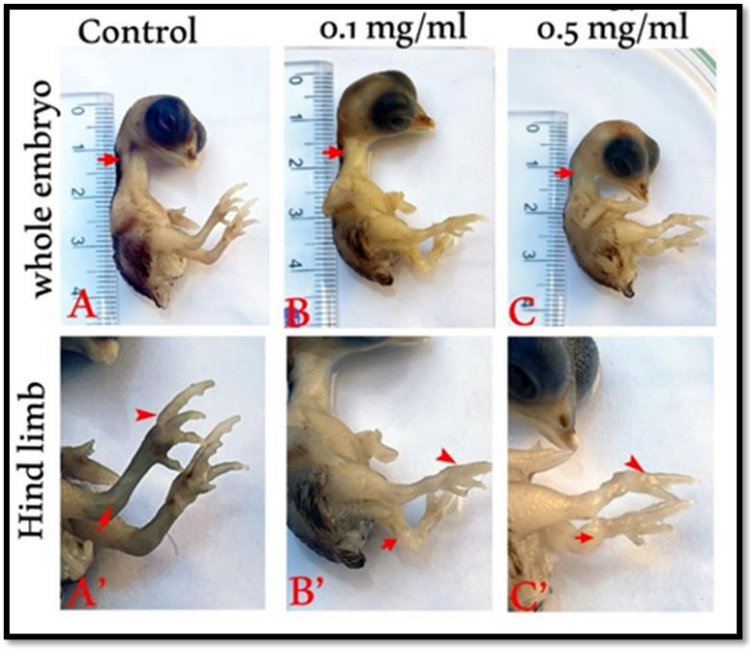

Figure 1 Effect of injection 0.1 mg/ml and 0.5 mg/ml of nicotine on developing chicken embryo at HH36. showed a lateral view of developing embryos at HH36, A control, B treated with 0.1 mg/ml and c treated with 0.5 mg/ml, A' hind limb of developing embryos HH36, B' the treatment with nicotine at concentration 0.1 Mg/ml and c' treatment nicotine at concentration 0.5Mg/ml.

The effect of nicotine on long bone ossification

Figure (2) below shows an effect of nicotine on chicken embryos injected at day zero and collected at stages HH36. It showed that the control group, treated with nicotine 0.1 Mg/ml and a group treated with nicotine 0.5 Mg/ml in stage HH36 were presented in (Fig 2 A and A') and (Fig 2 B and B') (Fig 2 C and C') consecutively. Alizarin red dye was used to determine the amount of calcium deposited in the bone and both controls were also observed to grow normally, (Fig 2 A) represents a control group and has no abnormalities, embryonic development is shown with the limbs present, and the distal parts of each wing and leg are relatively longer, Leg length in control 2.5 cm.

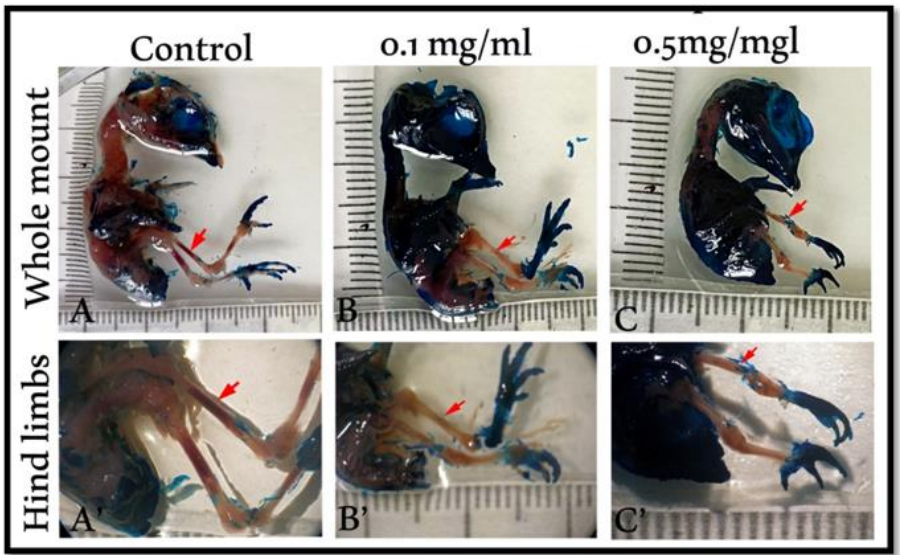

Figure 2 Effect of injection 0.1 mg/ml and 0.5 mg/ml of nicotine on ossification of long bone in chicken embryo at HH36 showed lateral view of developing embryos at HH36, A whole mount of developing embryos HH36, B treated with 0.1 mg/ml and c treated with 0.5 mg/ml, A' hind limb of developing embryos HH36, B' the treatment with nicotine at concentration 0.1 Mg/ml and C' treatment nicotine at concentration 0.5Mg/ml.

Figurer (2 A') the hind limbs of the legs were growing normally, as well as the length of the normally developing fingers, and the appearance of red alizarin dye in the thigh area is evidence of ossification in the hind limbs. Embryos treated with 0.1 mg/ml nicotine group (Fig 2 B), embryos showed developmental delay in head and legs, and it was observed that the external shape of the fetus differed from that of the control Leg length in control 2 Cm.(Fig 2 B') in which embryos showed abnormalities when compared to control in the hind limbs of the tibia and was growing abnormally and the presence of length deformity in the toes and the absence of alizarin red dye in the femoral region evidence of the absence of ossification in the hind limbs. While embryos treated with 0.5 mg/mL nicotine group (Fig 2 C), embryos showed growth retardation in head and legs size was small, leg length in control 1.5 cm. while (Fig 2 C') group, embryos showed very abnormally when compared to the control in the hind limbs of the leg and was growing abnormally and the presence of a large deformity in the toes as well as the length of the abnormally growing fingers are much smaller when compared to the control and the absence of alizarin red dye in the thigh area is evidence of the absence of ossification in the hind limbs.

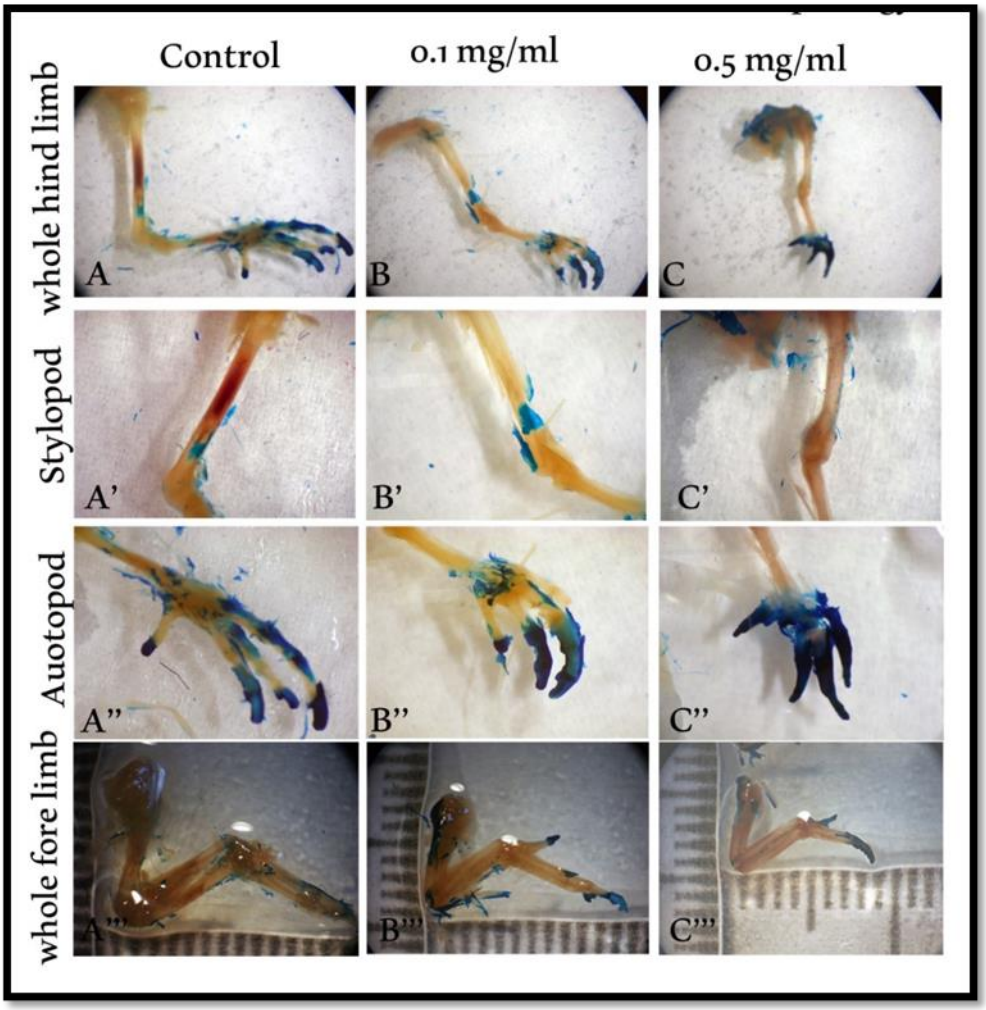

Figure 3 Effect of injection 0.1 mg/ml and 0.5 mg/ml of nicotine on developing embryo chicken at HH36. showed lateral view of developing embryos at HH36, (A) whole hind limb of developing embryos HH36 , (B) treated with 0.1 mg/ml and c treated with 0.5 mg/ml, (A') stylopid of developing embryos HH36 , (B') the treatment with nicotine at concentration 0.1 Mg/ml and (C') treatment nicotine at concentration 0.5Mg/ml, (A") Autopod of developing embryos HH36, (B") the treatment with nicotine at concentration 0.1 Mg/ml and (C") treatment nicotine at concentration 0.5Mg/ml, A"' whole fore limb of developing embryos HH36 , (B"') the treatment with nicotine at concentration 0.1 Mg/ml and (C"') treatment nicotine at concentration 0.5Mg/ml.

The study found that nicotine injections in chicken embryos can cause premature birth, delayed growth, and slowed skeletal growth. Higher nicotine concentrations led to more deaths, birth defects, and slowed growth. Nicotine altered bone development, reduced blood flow to bones, and reduced calcium absorption. This highlights the importance of proper nutrition and dietary intake in chicken embryos. The same result were observed by study done this year[13] they found that Statistically smaller length and ossification area, as well as a lower percentage

of ossification, were observed in the scapula, humerus, radius, and ulna bones of the LDN and HDN groups compared to the control group ($p<0.05$).

Conclusion.

Nicotine, a drug mimicking acetylcholine, is a major cause of congenital malformations during pregnancy. Its teratogenic effects on embryos are evident during the second trimester of pregnancy. Current study using chicken embryos indicated that nicotine exposure can lead to growth retardation, limb defects, and delayed bone ossification.

References

[1] R. L. Brent, "Environmental causes of human congenital malformations: the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors," *Pediatrics*, vol. 113, pp. 957-968, 2004.

[2] S. Chaabane and A. Bérard, "Epidemiology of major congenital malformations with specific focus on teratogens," *Current drug safety*, vol. 8, pp. 128-140, 2013.

[3] K. E. Farsalinos, N. Yannovits, T. Sarri, V. Voudris, and K. Poulas, "Nicotine delivery to the aerosol of a heat-not-burn tobacco product: comparison with a tobacco cigarette and e-cigarettes," *Nicotine and Tobacco Research*, vol. 20, pp. 1004-1009, 2018.

[4] D. B. Vaupel, S. R. Tella, D. L. Huso, V. O. Wagner, A. G. Mukhin, S. I. Chefer, *et al.*, "Pharmacological and toxicological evaluation of 2-FA-85380, a ligand for imaging cerebral nicotinic acetylcholine receptors with positron emission tomography," *Journal of Pharmacology and Experimental Therapeutics*, 2004.

[5] R. Wickstrom, "Effects of nicotine during pregnancy: human and experimental evidence," *Current neuropharmacology*, vol. 5, pp. 213-222, 2007.

[6] J.-X. Shen, D. Qin, H. Wang, C. Wu, F.-D. Shi, and J. Wu, "Roles of nicotinic acetylcholine receptors in stem cell survival/apoptosis, proliferation and differentiation," *Current Molecular Medicine*, vol. 13, pp. 1455-1464, 2013.

[7] D. S. Lambers and K. E. Clark, "The maternal and fetal physiologic effects of nicotine," in *Seminars in perinatology*, 1996, pp. 115-126.

[8] I. Peate, "Anatomy and physiology, 5. The musculoskeletal system," *British Journal of Healthcare Assistants*, vol. 12, pp. 6-9, 2018.

[9] M. J. Glimcher, "Bone: nature of the calcium phosphate crystals and cellular, structural, and physical chemical mechanisms in their formation," *Reviews in mineralogy and geochemistry*, vol. 64, pp. 223-282, 2006.

[10] X. Wang, H. C. Schröder, and W. E. Müller, "Amorphous polyphosphate, a smart bioinspired nano-/bio-material for bone and cartilage regeneration: towards a new paradigm in tissue engineering," *Journal of Materials Chemistry B*, vol. 6, pp. 2385-2412, 2018.

[11] Ż. Ciosek, K. Kot, D. Kosik-Bogacka, N. Łanocha-Arendarczyk, and I. Rotter, "The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue," *Biomolecules*, vol. 11, p. 506, 2021.

[12] O. Karmach, J. V. Madrid, S. Dasgupta, D. C. Volz, and N. I. Zur Nieden, "Embryonic exposure to cigarette smoke extract impedes skeletal development and evokes craniofacial defects in zebrafish," *International journal of molecular sciences*, vol. 23, p. 9904, 2022.

[13] K. Doğan, M. Nisari, A. Payas, T. Ertekin, H. Güler, and Ö. Al, "Effects Of Folic Acid Versus Nicotine On Bone Development," *Ahi Evran Medical Journal*, vol. 8, pp. 48-55, 2024.

[14] S. S. A. Abukhadir, N. Mohamed, S. Makpol, and N. Muhammad, "Effects of Palm Vitamin E on Bone-Formation-Related Gene Expression in Nicotine-Treated Rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, p. 656025, 2012.