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Ameloriate Effect of Glucose Monohydrate on Nicotine Sulfate-induced Toxicity and Teratogenicity in Xenopus embryos: an Experimental Study

Ayper Boga Pekmezekmek[1], Mustafa Emre[2], Erdal Tunç[3], Yasar Sertdemir[4]*

[1]*Department of Physiology, School of Medicine, Cukurova University, Adana, Türkiye*
https://orcid.org/0000-0002-3028-605X

[2]*Department of Biophysics, School of Medicine, Cukurova University, Adana, Türkiye*
https://orcid.org/0000-0001-9897-6674

[3]*Department of Medical Biology, School of Medicine, Artuklu University, Mardin, Türkiye*
https://orcid.org/0000-0003-4964-1004

[4]*Department of Biostatistics, School of Medicine, Cukurova University, Adana, Türkiye*
https://orcid.org/0000-0003-4455-3590

[*]*corresponding author's e-mail: aypbog@cu.edu.tr*

Abstract: It is well documented that nicotine causes low birth weight, preterm birth, pregnancy difficulties, lower fertility, inhibition of spermatogenesis, and decreased steroidogenesis and potassium channels conductance of Xenopus oocytes. Lung cancer is the most well-known adverse impact of nicotine. This work used a 96-hour FETAX test to examine how concurrent administration of glucose monohydrate modifies the effects of exposure to nicotine, nicotine sulfate, and/or glucose on ion channels and membrane potential in Xenopus leavis embryos at an early stage of development. In-vitro fertilised embryos were treated with nicotine and glucose alone or in combination for this aim, and the effects of those treatments were then assessed for potential teratogenic effects. At the conclusion of the FETAX technique, the ratios of healthy, abnormal, and dead embryos were calculated, and the length of embryos in each treatment group was assessed. The ratios of abnormal and dead embryos were considerably higher with nicotine treatment alone compared to controls. Compared to the results of the nicotine-alone treatment group, the ratio of aberrant embryos was marginally reduced by concurrent glucose and nicotine therapy. In contrast, the ratio of normal embryos was raised. Additionally, treatments with glucose, nicotine, and Nic+Glu significantly altered the resting membrane potentials of fertilised oocytes ($p < 0.001$). Our findings indicated that the simultaneous treatment groups that also received glucose had a protective impact on embryos. Such structured, more sophisticated research is required to confirm these findings.

Keywords: D(+) glucose monohydrate, embryo-teratogenicity, FETAX, nicotine sulfate, Xenopus laevis

1. Introduction

The main chemical component of tobacco smoke, nicotine, has detrimental impacts on how an embryo develops overall. As a result, smoking during pregnancy is linked to low birth weight, early birth, and neonatal morbidity and mortality in humans (Nelson & Taylor 2001, Wicström 2007, Machaalani et al. 2014). According to Oyeyipo et al. (2011), it also results in irregular menstruation, difficult pregnancies, and lower fertility in women. Emre et al. (2021) examined the effects of nicotine therapy on human fetal cells in a study. Their findings demonstrated that 25 ng/mL nicotine administration had a negative impact on human amniocytes and increased the ratio of apoptotic cells.

In a different study, 40 male and twenty-five female rats were used (Oyeyipo et al. 2011). The groups received nicotine treatments for 30 days at doses of 0.5 and 1.0 mg/kg body weight, respectively, while the control rats received 0.2 ml/kg of ordinary saline as a placebo. The study demonstrated that nicotine use has a dose-dependent detrimental effect on sperm features and that male rat fertility is improved by quitting smoking.

Numerous earlier investigations looked into the potentially negative effects of nicotine on the embryonic development of different model organisms and possible treatments. Such a study examined whether – carotene has an ameliorative impact against nicotine-induced increased reactive oxygen species (ROS), which causes embryonic stress in mouse embryos. They concluded that – carotene may be able to shield the embryos from nicotine's negative effects and promote healthy embryonic development. In a different study, Lin et al. (2012) looked into the anti-teratogenic properties of Resveratrol, a phytoalexin found in many different plants, including raspberries, grapes, peanuts, pistachios, and mulberries, against nicotine-induced teratogenesis in mouse embryos. They demonstrated how Resveratrol's antioxidative and anti-apoptotic properties exert a protective effect against nicotine-induced teratogenesis. Mammalian embryos drastically alter their energy metabolism during the start of blastocyst formation by beginning to use glucose instead of highly oxidised pyruvate. Glucose transport proteins signal this key transition.

Following that alternation, the survival and well growth of the embryo in utero depend on an appropriate supply of glucose from the mother to embryonic compartments. Due to the low level of glucose synthesis in embryos, it is crucial to maintain a steady supply of this essential substrate to support healthy embryo growth in utero (Korgun et al. 2001). According to a study, glucose may have teratogenic effects in a dose-dependent manner. In that investigation, embryos injected with 1300 mg/L of glucose displayed open neural tubes, but postimplantation embryos injected with 800 and 500 mg/L of glucose displayed smaller abnormalities (Torchinsky et al. 2003). Teixidó et al. (2013) looked at how eight substances, including glucose, affected the growth of zebrafish embryos. They investigated if glucose dosages ranging from 1 to 100 mM had teratogenic effects. All glucose exposure levels did not result in growth retardation or teratogenic consequences in developing zebrafish embryos. Another significant study examined the impact of the glucose transporter xGLUT1 on the growth of Xenopus embryos. According to this study (Suzawa et al. 2007), xGLUT1 transporters are critical for cell mobility during Xenopus gastrulation.

Additionally, research has shown that nicotine negatively affects glucose metabolism (Kennedy et al. 2017, Becker & Martin 1971, Eckstein et al. 1997). According to a related study, nicotine treatment during the early stages of embryonic development drastically reduced the levels of the GLUT1 transporter (55 kilodalton isoform) in the brains of chick embryos (Eckstein et al. 1997). The findings of certain research (Manos et al. 1991, Yeh et al. 2001) lead us to believe that glucose may have the ability to play a protective function against toxicants via G6PD enzyme activity because glucose is recognised as one of the modulators implicated in G6PD induction.

Therefore, the current investigation aimed to determine if the ideal concentration of D-glucose can reduce the negative effects of nicotine sulfate on Xenopus embryos. We utilised the FETAX (Frog Embryos Teratogenesis Assay: Xenopus) 96-hour whole embryo developmental toxicity screening test. Additionally, we evaluated their membrane potentials while D-glucose and nicotine were being administered concurrently to fertilised Xenopus laevis oocytes.

2. Material and Method

2.1. Study Plan

Between May 2017 and April 2018, this experimental investigation was conducted at the physiology section of the Medical Faculty of Cukurova University in Adana, Turkey. The American Society for Testing Materials' 2017 Standard Guide and the Declaration of Helsinki's 1995 (as revised in Brazil in 2013) guiding principles were completely adhered to in the execution of all processes and methodologies utilised in this investigation. Additionally, the Ethics Committee of the University of Cukurova granted their clearance for this study (04.24.2017/No:4).

To examine the teratogenic effects of various chemicals on Xenopus embryos, we employed the frog embryo teratogenesis assay-Xenopus (FETAX). When examining the detrimental effects of some environmental contaminants on the early stages of frog embryonic development, the FETAX test has been widely employed (Fort et al. 2000). In essence, it is an examination of organogenesis, a remarkably conserved process in the animal phylogeny. Several similarities exist between the first 96 hours of Xenopus embryonic development and human embryonic development. To identify probable teratogens and developmental toxicants for humans, FETAX results applied to frog embryos might also be helpful (Fort et al. 2000, Vismara et al. 2000).

In particular, the FETAX test outcomes from numerous investigations demonstrated an 89% concordance rate with those from mouse teratogenicity tests. Frog larvae length is a crucial parameter that assesses the outcome of the FETAX bioassay and offers insight into the teratogenic effects of a certain chemical (Fort et al. 2000, Dawson & Bantle 1987, Nieuwkoop & Faber 1994). Because they are simple to fertilise ex-utero and serve as excellent models for eukaryotic cells, Xenopus eggs were used for teratogenicity investigations. Additionally, an atlas depicting the stages of Xenopus development was previously used for comparison (Nieuwkoop & Faber 1994, Eide et al. 2000).

2.2. Test Materials

The following items were purchased: FETAX solution, nicotine sulfate, human chorionic gonadotropin (Pregnyl, 5000 IU, Organon), follicle-stimulating hormone (Serono), and DeBoers Tris (DBT) (Sigma). The doses of nicotine sulfate (nicotine, Nic) and D (+) glucose (Glucose, Glu) (800 mg/dL and 25 ng/mL, respectively) were chosen based on those used in prior investigations (Luck et al. 1985, Torchinsky et al. 2003). Petri-containing samples were initially supplemented with nicotine and glucose (Torchinsky et al. 2003, Demirhan et al. 2011).

2.3. Xenopus Leavis Maintenance and In Vitro Fertilisation Techniques

Turkey's Cukurova University School of Medicine's Physiology Department Adana provided mature Xenopus leavis (African Clawed Frogs). Animals were stored in 95-60-44 cm^3 aquariums, maintained at a constant temperature of 23°C (±2°C), and alternated between 12 hours of illumination and 12 hours of darkness. They were given access to food at any time (Boga et al. 2015). In vitro fertilisation of Xenopus eggs was carried out using techniques Lindi et al. (2001) outlined. Eggs with disorganised segmentation were taken out and thrown away. Using the standard tables, embryos between midblastula (stage 8) and early gastrula (stage 11), were chosen and used for the FETAX procedures (Nieuwkoop & Faber 1994, Boga et al. 2015).

2.4. Application of FETAX Procedure

Utilised fertilised embryos were made from the oocytes and sperm of six Xenopus frogs (three male and three female). There was one control group and seven research groups used in the studies. Tables 1 and 2 show that the study groups were divided into Nic, Glu, Glu+Nic, and therapy groups. The embryos in the first two experimental groups were exposed to nicotine (25 ng/mL) and glucose (800 mg/dL) alone, while the embryos in the third group were exposed to mixtures of those substrates. 540 assay embryos (three study groups), 180 control embryos, and a total of 720 embryos were used. Each study group had 60 embryos, but because all studies were conducted three times, each group had 180 embryos. The three Petri plates, each with 20 embryos, were used to test the effects of various chemicals on the assay embryos of each study group. Petri plates underwent the necessary procedures and were incubated at 23(±1)°C. At 24, 48, and 72 hours, the FETAX solutions in the Petri plates were changed. The embryos and tadpoles were photographed and assessed based on their head-to-tail lengths after the FETAX procedure's 96-hour incubation period. The length of embryos was measured using a 10-magnification Olympus SZ-61 type ocular micrometre. It was also established how many tadpoles were alive and viable, and after that, the tadpoles were fixed in a 3.0% formalin solution (pH 7.0).

Embryos were inspected utilising a binocular dissecting microscope. The absence of heartbeats (at the 72nd and 96th hours), structural integrity, irritability (at the 24th and 48th hours), and skin colour were utilised as indicators of death. It was also assessed how many deformed embryos survived across all dishes. To distinguish between normal and abnormal embryos, researchers employed the Xenopus laevis normal table and the ASTM Standard Guide (2017) (Yamaguchi & Shinagawa 1989, Nieuwkoop & Faber 1994, Lindi et al. 2001).

2.5. Measurements of Membrane Potential and Oocyte Study Setup

Glass microelectrodes loaded with 3M KCI and of 20–30 M tip resistances were used to test membrane potentials. The reference electrode was a 3M KCl-filled Ag-AgCl agar-jel bridge. According to the procedure described by Stuart et al. (2006), membrane potential measurements were carried out, and the setup is shown in Figure 1. Oocytes and fertilised eggs were examined with a stereo-zoom microscope. The potential difference between the reference and penetrating electrodes was denoted by the abbreviation Vmem. At room temperature, membrane potentials were measured. We measured the membrane potentials of resting (RMPs) and fertilised (FMPs) eggs.

2.6. Analytical Statistics

While continuous data were summed up as mean, standard deviation, and 95% CI, categorical variables were reported as numbers and percentages. For continuous data, the Kolmogorov-Smirnov test was employed to validate the normality of the distribution. The Chi-square test was performed to compare categorical variables between groups. The embryo length of the groups was compared using a one-way ANOVA test. Depending on the homogeneity of the variances, the Tukey and Games&Howell tests were utilised for multiple comparison correction. The statistical program IBM SPSS Statistics Version 19.0 was used for all analyses. (2010) The threshold for statistical significance was set at 0.05.

3. Result

3.1. Normal abnormal and death ratio

In the study, ratios of normal, abnormal, and fatalities are compared in Table 1.

Table 1. Ratios of normal, abnormal, and dead embryos for control and all treatment groups

Group	Normal(%)	Abnormal(%)	Death(%)	Total(%)
Control	175(97%)	3(2%)	2(1%)	180(100%)
Glu	175(97%)	5(3%)	0(0%)	180(100%)
Nic	0(0%)#	153(85%)#	27(15%)#	180(100%)
Nic+Glu	45(25%)#	135(75%)#	0(0%)#	180(100%)

[#] *indicates a significant difference from control ratios* ($p < 0.001$)

Table 1 displays the normal, abnormal, and dead embryo ratios for all study and control groups. The ratios of normal embryos were 97 and 97 for the control and glucose therapy groups, respectively. Compared to control ratios, the glucose therapy group's normal, abnormal, and dead embryo ratios did not differ substantially ($p > 0.05$). In the nicotine treatment group, the ratios of malformed and dead embryos were 85% and 15%, respectively. There were no deaths in the concurrent therapy (Glu+Nic) group, although there were 75% aberrant and 25% normal embryos ($p < 0.001$).

3.2. Abnormality

In abnormal and dead embryos, significant malformations were discovered. Microphthalmia, microcephaly, incomplete eyes (head malformations), gut malrotation (trunk malformation), kinked and coiled tails (tail malformations), and mild edema were some of the deformities seen in abnormal and dead embryos (Figure 1).

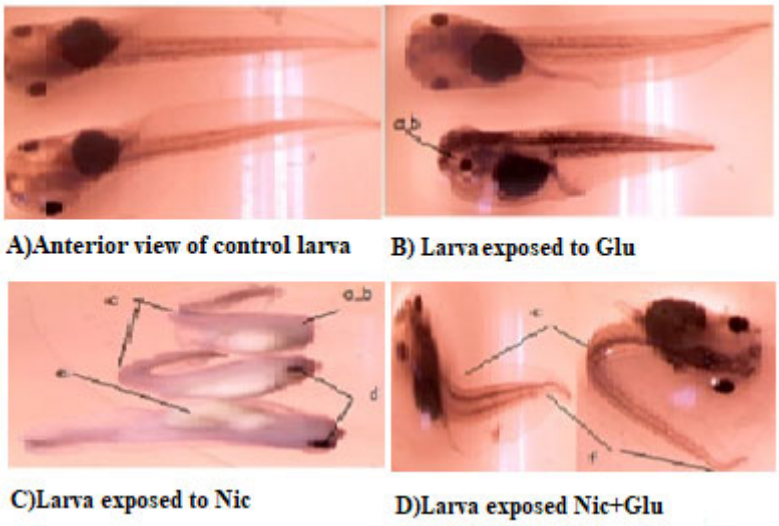

Fig. 1. Appearances of exemplar larvas for all control and treatment groups. Larvas-treated with Glu, Nic, and combinations of Glu+Nic. a) microphthalmia b) microcephaly c) curled tail d) incomplete eye e) abnormal gut f) kinked tail

3.3. Values of Growth

Table 2 displays the growth values for all embryo groups. Upper-lower bounds for control embryos were 7.79 mm and 7.72 mm, respectively, with a mean growth value of 7.72 mm. The mean growth values for the Nic, Glu, and Glu+Nic therapy groups were 3.73 mm, 7.04 mm, and 6.59 mm, respectively. Upper-lower length values for those treatment groups ranged between 7.72-3,83 and 7.65-3,63 mm.

Table 2. Nic, Glu, and Glu+Nic treated embryos showed significant decreases in length, with more dramatic decreases in embryos exposed to Nic

Group	Mean (mm)	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Control	7.72	3.65	7.65	7.79
Glu	7.04*	3.63	6.97	7.11
Glu+Nic	6.59*	3.63	6.52	6.66
Nic	3.73*	5.13	3.63	3.83

*indicates a significant difference from control values ($p < 0.05$)

Compared to control values, those embryo groups' growth values showed a significant decline ($p < 0.05$). The effects of glucose drastically reduced embryo length measurements.

3.4. Membrane potential measurements

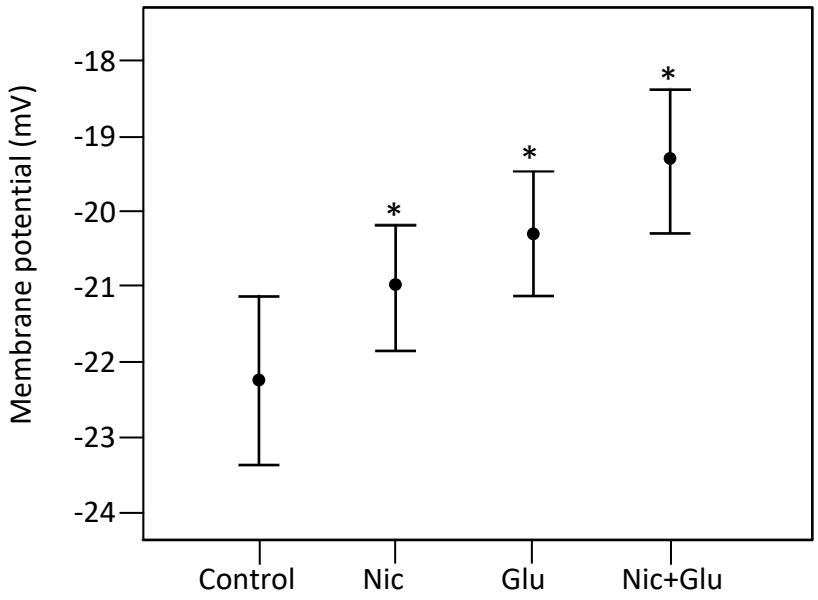

Fig. 2. Nic, Glu, and Nic+Glu treatments depolarised the resting membrane potentials of fertilised oocytes
* indicates a significant difference from fertilised controls ($p < 0.05$)

Figure 2 displays the outcomes of the measurements of membrane potentials. In both treatment groups, the given chemicals markedly depolarised the membrane potentials of fertilised oocytes ($p < 0.05$).

4. Discussion

Treatment with 800 mg/dL of glucose resulted in a 3% aberrant embryo ratio and no deaths (0%), ratios that were not statistically different from control ratios. Embryos were brought to culture from 8-day pregnant mice in a prior investigation by Torchinsky et al. (2003). 97%, 68%, and 37% of those embryos underwent maldevelopment when exposed to glucose levels of 1300, 800, and 500 mg/dL, respectively. In comparison to medium containing glucose doses of 1300 mg/dL, it was found that the proportion of deformed mouse embryos was lower in media containing glucose doses of 800 and 500 mg (Torchinsky et al. 2003). According to Kirchick et al. (1982), healthy mice had fasting glucose levels between 80 and 100 mg/dL; hence the extremely high glucose concentrations caused a higher proportion of embryos to be deformed. Cellular processes that may contribute to high-dose glucose-induced toxicity include methylglyoxal production and glycation (AGE), glucose autoxidation, sorbitol formation, apoptosis, and inhibition of phosphorylation (Kirchick et al. 1982, Viñals et al. 1999, Torchinsky et al. 2003, Poitout et al. 2006, Canimoğlu & Rencüzoğullari 2006). It is conceivable that the toxic effects produced by those cellular processes, which later result in maldevelopments, are most detrimental to developing embryos.

Treatment with 800-mg/dL glucose resulted in 3% malformed embryos in the current investigation but no deaths, suggesting that this glucose concentration had fewer negative effects than those reported in the literature. However, in our investigation, an 800-mg/dL glucose dose significantly decreased embryo length (7.04 mm vs. 7.72 mm) compared to the control value. Torchinky et al. (2003) observed that mouse embryos produced in media with glucose levels of 1300 and 800 mg/dL showed a significant decrease in the number of somites, which is consistent with our findings. D-glucose treatments resulted in developmental delays in zebrafish larvae, supporting these findings (Teixidó et al. 2010). Dissimilar outcomes from several researches can be related to variations in the animal models employed, the protocols used, and the doses used. Numerous research using various study methods have documented the harmful effects of nicotine on a range of species and cell types (Hammer et al. 2011, Feng et al. 2014). According to a study, exposure to nicotine-containing e-cigarette liquids may be harmful to Xenopus eggs that are still growing. According to the findings of that study (Kennedy et al. 2017), nicotine worsened the negative effects of other e-liquid ingredients. The current investigation demonstrated that nicotine treatment dramatically raised the ratio of abnormal and dead embryos, which is consistent with these findings. Our previous study found that nicotine alone and 900 MHz RF-EMR and nicotine combined treatments significantly increased apoptosis/necrosis ratios in human fetal cells (Boga et al. 2015). According to the current investigation results, Glu+Nic concurrent treatments greatly raised the ratio of normal embryos and zero embryo deaths while only marginally decreasing the percentage of defective embryos (from 85% to 75%). It indicates that the harmful effects of nicotine on embryos were reduced when glucose was administered concurrently with nicotine treatments.

The pentose phosphate pathway, a crucial molecular shunt that makes glucose an essential necessity for embryo development, is responsible for glucose's protective effect against nicotine's negative effects (Zhu & Zernicka-Goetz 2020). The pentose phosphate pathway, which includes the key enzyme glucose-6-phosphate dehydrogenase (G6PD), is essential for glucose metabolism. Ribose-5-phosphate, mainly needed for nucleic acid synthesis and cell proliferation, is the end product of the pentose phosphate pathway (Jeng et al. 2013). One of the most important antioxidative enzymes for development, G6PD guards the embryo against the harmful effects of endogenous oxidative stress, xenobiotic-induced ROS production, DNA damage, and teratogenesis (Nicol et al. 2000). Therefore, there is a straightforward antagonistic relationship between nicotine's ability to cause excessive ROS generation and G6PD's ability to prevent ROS creation (Lin et al. 2013). We believe that this antagonistic relationship may also be responsible for glucose's protective impact against nicotine-induced damage in Xenopus embryos. Carefully planned molecular experiments are required to support this interpretation of the findings.

On the other hand, aberrant cation flow brought on by electromagnetic fields can alter the biochemical characteristics of cell membranes and impair cation channel performance, particularly voltage-gated ion channel activity. According to Davis et al. (2002) and Kiselyov and Muallem (2016), such a deficiency in ion channel function might disrupt various cellular processes, causing DNA damage and oxidative stress and indirectly causing teratogenic consequences and related illnesses. DNA synthesis, mitosis, and general cell cycle progression events – all of which are factors in cell proliferation and differentiation processes – are strongly connected with the levels of resting membrane potential in cells. Positions of cells along this scale of membrane potentials often correspond to their proliferative potentials. The resting membrane potentials of various cell types range widely (typically from 10 mV to 90 mV). As a result, the membrane potential value is a crucial factor in determining how a chemical will affect cells (Sundelacruz et al. 2009). As frog embryos develop, some changes can be seen in the membrane potential of neural cells. Early frog embryos exhibit a distinctive, intense hyperpolarisation in the neural tube-lining cells; disruption of this spatial gradient of the transmembrane potential (Vmem) reduces or eliminates the expression of early brain markers and results in anatomical brain mispatterning, including absent or malformed regions. These disastrous effects may result from any disruption in the operation of voltage-gated calcium signalling and gap-junctional communication (Pai et al. 2015). In ventral cells outside of the brain, hyperpolarisation of the transmembrane potential (Vmem) in 2015 was found to long-term induce upregulation of neural cell proliferation. The resting membrane potentials of fertilised oocytes were significantly altered by the glucose, nicotine, and Nic+Glu treatments in the current investigation (Figure 2, $p < 0.001$).

The depolarisation of oocytes subjected to nicotine and glucose. According to research that supports this finding, glucose increased the ATP/ADP ratio in rat pancreatic islet cells, shut KATP channels, and depolarised the cell membrane. Because it has been noted that endogenous gradients of resting potential direct embryonic brain cells for patterning through Notch signalling and regulation of proliferation (Pai et al. 2015), it may be assumed that glucose-induced membrane depolarisation resulted in a reduction in embryo length. The Ion channel and membrane potential effects of nicotine have received little research attention (Wang et al. 2000, Bébarová et al. 2017). The effects of nicotine on Xenopus oocyte Kv4.3 and Kv4.2 potassium channels were

examined in this work (Wang et al. 2000). That study showed that nicotine significantly suppressed the potassium channel Kv4 channel current, rapidly inactivates potassium channels. Nicotine decreased the channel conductance at the single-channel level. Here, we propose a link between Xenopus embryonic head defects such as microphthalmia, microcephaly, and incomplete eyes and nicotine sulfate-induced membrane depolarisation (Figure 2).

5. Conclusion

The findings of nicotine treatments adopted clear evidence for the teratogenic action of nicotine and its mixtures, as they resulted in high ratios of deformities and fatalities throughout the development of Xenopus embryos. It will be helpful to find potential attenuators of nicotine action if the data show that concurrent D-Glucose treatments reduced the negative effects of nicotine on embryonic development. This idea needs to be verified, and it provides a foundation for future research on this crucial topic. Our findings may also be significant for humans because the results from frogs (using FETAX) are 89% comparable with the results from mammals. The findings of the current study are so crucial for pregnant moms who are exposed to tobacco smoke. Additional research is required to corroborate these findings.

References

American Society for Testing and Materials (ASTM International). Standard guide for conducting the frog embryo teratogenesis assay – Xenopus (FETAX). 2017 [cited 2022 Oct 27]. Available from: http://www.astm.org/e1439-98.html

Bébarová, M., Matejovič, P., Švecová, O., Kula, R., Šimurdová, M., Šimurda, J. (2017). Nicotine at clinically relevant concentrations affects atrial inward rectifier potassium current sensitive to acetylcholine. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *390*, 471-481. https://doi.org/10.1007/s00210-017-1341-z

Becker, R.F., Martin, J.C. (1971). Vital Effects of Chronic Nicotine Absorption and Chronic Hypoxia Stress During Pregnancy and the Nursing Period. *American Journal of Obstetric and Gynecology*, *110*(4), 522-533. https://doi.org/10.1016/0002-9378(71)90693-4

Boga, A., Emre, M., Sertdemir, Y., Akillioglu, K., Binokay, S., Demirhan, O. (2015). The effect of 900 and 1800 MHz gsm-like radiofrequency irradiation and nicotine sulfate administration on the embryonic development of Xenopus laevis. *Ecotoxicology and Environmental Safety*, *113*, 378-390. https://doi.org/10.1016/j.ecoenv.2014.12.020

Canimoğlu, S., Rencüzoğullari, E. (2006). The Cytogenetic Effects of Food Sweetener Maltitol in Human Peripheral Lymphocytes. *Drug and Chemical Toxicology*, *29*(3), 269-78. https://doi.org/10.1080/01480540600651600

Davis, G., Edwards, R., Hille, B., Lewis, J., Morgan, D., Raff, M., Roberts, K. (2002). *Membrane transport of small molecules and the electrical properties of membranes*. In: Alberts B, Johnson A, Lewis J, Raff M, Roberts K, et al. (Ed), Molecular Biology of the Cell. 6th ed., New York, NY, USA: Garland Science; p. 597-641.

Dawson, D.A, Bantle J.A. (1987). Development of a reconstituted water medium and preliminary validation of the frog embryo teratogenesis assay – Xenopus (FETAX). *Journal of Applied Toxicology*, *7*(4), 237-244. https://doi.org/10.1002/jat.2550070403

Demirhan, O., Demir, C., Tunç, E., Inandıklıoğlu, N., Sütcü, E., Sadıkoğlu, N., Ozcan B. (2011). The genotoxic effect of nicotine on chromosomes of human fetal cells: the first report described as an important study. *Inhalation Toxicology*, 23, 829-834. https://doi.org/10.3109/08958378.2011.617398

Eckstein, L.W., Shibley, Jr I.A., Pennington, J.S., Carver, F.M., Pennington, S.N. (1997). Changes in Brain Glucose Levels and Glucose Transporter Protein Isoforms in Alcohol-or Nicotine-Treated Chick Embryos. *Developmental Brain Research*, *103*(1), 59-65. https://doi.org/10.1016/S0165-3806(97)00117-X

Eide, F.F., Eisenberg, S.R., Sanders, T.A. (2000). Electroporation-mediated gene transfer in free-swimming embryonic Xenopus laevis. *FEBS Letter*, *486*, 29-32. https://doi.org/10.1016/s0014-5793(00)02124-4

Emre, M., Boğa, A., Cetiner, S., Tunc, E., Demirhan, O. (2021). The effects of exposure to 900 MHz radiofrequency radiation and nicotine on the apoptotic ratio of human fetal cells. *Southeast European Journal of Immunology*, *4*, 1-7. https://doi.org/10.3889/seejim.2021.3249

Feng, J.H., Yan, Y.E., Liang, G., Liu, Y., Li, X., Zhang, B. (2014). Maternal and fetal metabonomic alterations in prenatal nicotine exposure-induced rat intrauterine growth retardation. *Molecular and Cellular Endocrinology*, *394*, 59-69. https://doi.org/10.1016/j.mce.2014.06.016

Fort, D.J., Stover, E.L., Farmer, D.R., Lemen, J.K. (2000). Assessing the predictive validity of frog embryo teratogenesis assay-Xenopus (FETAX). *Teratogenesis Carcinogenesis and Mutagenesis*, *20*, 87-98.

Hamirah, N.K., Kamsani, Y.S., Khan, N., Rahim, S.A., Rajikin, M.H. (2017). Effects of Nicotine and Tocotrienol-Rich Fraction Supplementation on Cytoskeletal Structures of Murine Pre-Implantation Embryos. *Medical Science Monitor Basic Research*, 23373-379. https://doi.org/10.12659/msmbr.905447

Hammer, T.R., Fischer, K., Mueller, M., Hoefer, D. (2011). Effects of cigarette smoke residues from textiles on fibroblasts, neurocytes and zebrafish embryos and nicotine permeation through human skin. *International Journal of Hygiene and Environmental Health*, *214*, 384-391. https://doi.org/10.1016/j.ijheh.2011.04.007

Heart, E., Corkey, R.F., Wickstrom. J.D., Shirihai, O.S., Corkey, B.E. (2006). Glucose-dependent increase in mitochondrial membrane potential, but not cytoplasmic calcium, correlates with insulin secretion in single islet cells. *American Journal of Physiology-Endocrinology and Metabolism*, *290*, E143-E148. https://doi.org/10.1152/ajpendo.00216.2005.

IBM SPSS Statistics. IBM SPSS Statistics for Windows, Version 19.0 Armonk, NY: IBM Corp. 2010. [cited 2018 Oct 20] Available from: https://www.ibm.com/products/spss-statistics

Jeng, W., Loniewska, M.M., Wells, P.G. (2013). Brain glucose-6-phosphate dehydrogenase protects against endogenous oxidative DNA damage and neurodegeneration in aged mice. *ACS Chemical Neuroscience*, *4*, 1123-1132. https://doi.org/10.1021/cn400079y

Kang, T., Zhao, S., Shi, L., Li, J. (2021). Glucose metabolism is required for oocyte maturation of zebrafish. *Biochemical and Biophysical Research Communications*, *559*, 191-196. https://doi.org/10.1016/j.bbrc.2021.04.059

Kennedy, A.E., Kandalam, S., Olivares-Navarrete, R., Dickinson, A.J.G. (2017). E-Cigarette Aerosol Exposure Can Cause Craniofacial Defects in Xenopus laevis Embryos and Mammalian Neural Crest Cells. *PLoS One*, *12*(9), e0185729. https://doi.org/10.1371/journal.pone.0185729.g004

Kirchick, H.J., Keyes, P.L., Frye, B.E. (1982). Restoration of the LH Surge and Ovulation by Insulin in Alloxan-Diabetic Immature Rats Treated with Pregnant Mare's Serum Gonadotrophin. *Acta Endocrinologica* (Copenhagen), *100*, 266-73. https://doi.org/10.1530/acta.0.1000266

Kiselyov, K., Muallem, S. (2016). ROS and intracellular ion channels. *Cell Calcium*, *60*, 108-114. https://doi.org/10.1016/j.ceca.2016.03.004

Korgun, E.T., Demir, R., Hammer, A., Dohr, G., Desoye, G., Skofitsch, G., Hahn, T. (2001). Glucose transporter expression in rat embryo and uterus during decidualisation, implantation, and early postimplantation. *Biology of Reproduction*, *65*(5), 1364-70. https://doi.org/10.1095/biolreprod65.5

Lin, C., Yon, J.M., Jung, A.Y., Lee, JG, Jung, K.Y., Kang, J.K., Lee, Beom Jun., et al. (2012). Resveratrol prevents nicotine-induced teratogenesis in cultured mouse embryos. *Reproductive Toxicology*, *34*(3), 340-346. https://doi.org/10.1016/j.reprotox.2012.05.097

Lin, C., Yon, J.M., Jung, Y.A., Lee, J.G., Jung, K.Y., Lee, B.J., et al. (2013). Antiteratogenic Effects of β-Carotene in Cultured Mouse Embryos Exposed to Nicotine. *Evidence-based Complementary and Alternative Medicine*, *2013*, 575287. https://doi.org/10.1155/2013/575287

Lindi, C, Montorfano, G., Rossi, F., Montorfano, G., Rossi, R., Gornati, R., Rizzo, AM. (2001). Effect of ethanol exposure on Xenopus embryo lipid composition. *Alcohol Alcohol*, *36*, 388-392. https://doi.org/10.1093/alcalc/36.5.388

Luck, W., Nau, H., Hansen, R., Steldinger, R. (1985). The extent of nicotine and cotinine transfer to the human fetus, placenta, and amniotic fluid of smoking mothers. *Developmental Pharmacology and Therapeutics*, *8*, 384-395. https://doi.org/10.1159/000457063

Machaalani, R., Ghazav, E., Hinton, T.,Waters, K.A., Hennessy, A. (2014). Cigarette smoking during pregnancy regulates the expression of specific nicotinic acetylcholine receptor (nAChR) subunits in the human placenta. *Toxicology and Applied Pharmacology*, *276*(3), 204-212. https://doi.org/10.1016/j.taap.2014.02.015

Manos, P., Nakayama, R., Holten, D. (1991). Regulation of glucose-6-phosphate dehydrogenase synthesis and mRNA abundance in cultured rat hepatocytes. *Biochemical Journal*, *276*, 245-250. https://doi.org/10.1042/bj2760245

Nelson, E.A., Taylor, B.J. (2001). International childcare practices study infant sleep position and parental smoking. *Early Human Development*, *64*, 7-20. https://doi.org/10.1016/S0378-3782(01)00165-7

Nicol, C.J., Zielenski, J., Tsui, L.C., Wells, P.G. (2000). An embryo-protective role for glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. *The FASEB Journal*, *14*, 111-127. https://doi.org/10.1096/fasebj.14.1.111

Nieuwkoop, P.D., Faber, J. (1994). Normal table of Xenopus laevis (Daudin). Newyork&London: Garland Publishing.

Oyeyipo, I.P., Raji, Y., Emikpe, B.O., Bolarinwa, A.F. (2011). Effects of nicotine on sperm characteristics and fertility profile in adult male rats: a possible role of cessation. *Journal of Reproduction & Infertility*, *12*(3), 201-7.

Pai, V.P., Lemire, J.M., Paré, J.F., Lin, G., Chen, Y., Levin, M. (2015). Endogenous gradients of resting potential instructively pattern embryonic neural tissue via Notch signaling and regulation of proliferation. *Journal of Neuroscience*, *35*, 4366-4385. https://doi.org/10.1523/JNEUROSCI.1877-14.2015

Poitout, V., Hagman, D., Stein, R., Artner, I., Robertson, P., Harmon, J.S. (2006). Regulation of the Insulin Gene by Glucose and Fatty Acids. *The Journal of Nutrition*, *136*(4), 873-6. https://doi.org/10.1093/jn/136.4.873

Sundelacruz, S., Levin, M., Kaplan, D.L. (2009). Role of membrane potential in the regulation of cell proliferation and differentiation. *Stem Cell Reviews and Reports*, *5*, 231-246. https://doi.org/10.1007/s12015-009-9080-2

Suzawa, K., Yukita, A., Hayata, T., Goto, T., Danno, H., Michiue, T., … Asashima, M. (2007). Xenopus glucose transporter 1 (xGLUT1) is required for gastrulation movement in Xenopus laevis. *The International Journal of Developmental Biology*, *51*(3), 183-90. https://doi.org/10.1387/ijdb.062230ks

Stuart, G.J., Palmer, L.M. (2006). Imaging membrane potential in dendrites and axons of single neurons. *Pflügers Archiv*, *453*, 403-410.

Teixidó, E., Piqué, E., Gómez-Catalán, J., Llobet, J.M. (2013). Assessment of developmental delay in the zebrafish embryo teratogenicity assay. *Toxicology In Vitro*, *27*, 469-478. http://dx.doi.org/10.1016/j.tiv.2012.07.010

Torchinsky, A., Brokhman, I., Shepshelovich, J., Orenstein, H., Savion, S., Zaslavsky, Z, Koifman, M.,…Toder, V. (2003). Increased TNF- Expression in Cultured Mouse Embryos Exposed to Teratogenic Concentrations of Glucose. *Reproduction*, *125*, 527-534. https://doi.org/10.1530/rep.0.1250527

Viñals, F., Gros, A., Testar, X., Palacín, M., Rösen, P., Zorzano, A. (1999). High Glucose Concentrations Inhibit Glucose Phosphorylation, but not Glucose Transport, in Human Endothelial Cells. *Biochimica et Biophysica Acta (BBA) – Molecular Cell Research*, *1450*(2), 119-129. https://doi.org/10.1016/s0167-4889(99)00035-x

Vismara, C., Battista, V.V., Vailati, G., Bacchetta, R. (2000). Paraquat induced embryotoxicity on Xenopus laevis development. *Aquatic Toxicology*, *49*, 171-179. https://doi.org/10.1016/s0166-445x(99)00080-6

Yamaguchi, Y., Shinagawa, A. (1989). Marked alteration at midblastula transition in the effect of lithium on the formation of the larval body pattern of Xenopus laevis. *Development, Growth & Differentiation*, *31*, 531-541. https://doi.org/10.1111/j.1440-169X.1989.00531.x

Yeh, G.C., Daschner, P.J., Lopaczynska, J., MacDonald, C.J., Ciolino, H.P. (2001). Modulation of glucose-6-phosphate dehydrogenase activity and expression is associated with aryl hydrocarbon resistance in vitro. *Journal of Biological Chemistry*, *276*, 34708-34713. https://doi.org/10.1074/jbc.M105680200

Wang, H., Shi, H., Zhang, L., Pourrier, M., Yang, B., Nattel, S., Wang, Z. (2000). Nicotine is a potent blocker of the cardiac a-type (+) channels. Effects on cloned kv4.3 channels and native transient outward current. *Circulation*, *102*, 1165-1171. https://doi.org/10.1161/01.cir.102.10.1165

Wickström, R. (2007). Effects of nicotine during pregnancy: human and experimental evidence. *Current Neuropharmacology*, *5*, 213-222. https://doi.org/10.2174/157015907781695955

Zhu, M., Zernicka-Goetz, M. (2020). Living a sweet life: glucose instructs cell fate in the mouse embryo. *Developmental Cell*, *53*, 1-2. https://doi.org/10.1016/j.devcel.2020.03.012