DOI: 10.22055/ivj.2025.545068.2841

Protective effect of ellagic acid on epididymal sperm profile changes and testicular tissue apoptosis in male rats receiving nicotine

Kaveh Khazaeel[1*], Abbas Sadeghi[2], Haifa Ali Hussein[3] and Javad Jamshidian[4]

[1] Associate Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran and Stem Cells and Transgenic Technology Research Center (STTRC), Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2] PhD Graduate in Comparative Anatomy and Embryology, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[3] Lecturer, Department of Anatomy and Histology, Faculty of Veterinary Medicine, University of Basrah, Basrah, Iraq
[4] Assistant Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Received: 04.09.2025 Accepted: 26.11.2025

Abstract

Nicotine (NC) is the major alkaloid in tobacco and has been strongly associated with male reproductive toxicity, mainly through mechanisms related to oxidative stress and apoptosis. Ellagic acid (EA), a naturally occurring polyphenol with well-documented antioxidant and anti-inflammatory properties, has been reported to counteract oxidative tissue damage in various experimental models. The present study investigated the protective effects of EA against NC-induced reproductive impairment in adult male Wistar rats. To do this, a total of twenty-four adult male Wistar rats were randomly divided into four groups (n = 6), including control group, EA group (60 mg/kg, orally), NC group (1 mg/kg, intraperitoneally), and NC combined with EA group. Following 30 days of treatment, the animals were euthanized, and testicular tissues were examined for gonadosomatic index (GSI), sperm quality parameters (count, motility, viability, and morphology), and expression levels of key apoptotic genes (Bcl-2, caspase-3, p53, and BAX). The administration of NC significantly reduced GSI, sperm count, motility, viability, and normal sperm morphology compared to the control group. Also, NC significantly increased the expression of p-53, caspase-3, and BAX genes and decreased Bcl-2 gene expression. Co-treatment with EA and NC significantly attenuated these adverse effects and improved reproductive parameters and apoptotic gene expression. In conclusion, these findings suggest that EA mitigates NC-induced reproductive toxicity in rats by reducing apoptosis, indicating its potential as a therapeutic agent for NC-related reproductive disorders.

Key words: Nicotine, Ellagic acid, Reproductive toxicity, Apoptosis, Testis

[*] **Corresponding Author**: Kaveh Khazaeel, Associate Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran and Stem Cells and Transgenic Technology Research Center (STTRC), Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: k.khazaeil@scu.ac.ir

Refrences

Aitken, R. J. (2020). Impact of oxidative stress on male and female germ cells: Implications for fertility. *Reproduction*, *159*(4), 189–201.

Alkully, T., El-Refaei, M. F., & Abdallah, E. A. (2025). Protective Effects of Ellagic Acid Against Arsenic-Induced Hepatotoxicity in Male Wistar Rats: Impact of Heme Oxygenase-1 Upregulation. *Food Science & Nutrition*, *13*(6), e70344.

ALTamimi, J. Z., AlFaris, N. A., Aljabryn, D. H., Alagal, R. I., Alshammari, G. M., Aldera, H., Yahya, M. A. (2021). Ellagic acid improved diabetes mellitus-induced testicular damage and sperm abnormalities by activation of Nrf2. *Saudi Journal of Biological Sciences*, *28*(8), 4300-4310.

Ansari, A., Fazeli, M., & Jalaei, A. (2022). Characterization of green-synthetized silver nanoparticles using Astragalus brachycalyx L. leaf extract and evaluation of its effect on diabetes in Wistar rats. *Iranian Veterinary Journal*, *18*(3), 5-17.

Aprioku, J. S., & Ugwu, T. C. (2015). Comparative evaluation of the impact of subacute exposure of smokeless tobacco and tobacco smoke on rat testis. *International journal of reproductive medicine*, *2015*(1), 676245.

Aslan, A., Beyaz, S., Gok, O., & Erman, O. (2020). The effect of ellagic acid on caspase-3/bcl-2/Nrf-2/NF-kB/TNF-α/COX-2 gene expression product apoptosis pathway: a new approach for muscle damage therapy. *Molecular biology reports*, *47*(4), 2573-2582.

Azad, F., Nejati, V., Shalizar-Jalali, A., Najafi, G., & Rahmani, F. (2019). Antioxidant and anti-apoptotic effects of royal jelly against nicotine-induced testicular injury in mice. *Environmental toxicology*, *34*(6), 708-718.

Benowitz, N. L., Bernert, J. T., Foulds, J., Hecht, S. S., Jacob III, P., Jarvis, M. J., Piper, M. E. (2020). Biochemical verification of tobacco use and abstinence: 2019 update. *Nicotine and Tobacco Research*, *22*(7), 1086-1097.

Bhattacharjee, A., Kulkarni, V. H., Chakraborty, M., Habbu, P. V., & Ray, A. (2021). Ellagic acid restored lead-induced nephrotoxicity by anti-inflammatory, anti-apoptotic and free radical scavenging activities. *Heliyon*, *7*(1).

Budin, S. B., Kho, J. H., Lee, J. H., Ramalingam, A., Jubaidi, F. F., Latif, E. S., ... & Mohamed, J. A. M. A. L. U. D. I. N. (2017). Low-dose nicotine exposure induced the oxidative damage of reproductive organs and altered the sperm characteristics of adolescent male rats. *The Malaysian journal of medical sciences: MJMS*, *24*(6), 50.

Cui, J., Wang, C., Zheng, Y., Zhang, Y., Luo, S., Ren, Z., Ding, G. (2025). Mechanisms and reversibility of nicotine-induced spermatogenesis impairment and DNA methylation changes. *Communications Biology*, *8*(1), 1053.

Das, S., Chakraborty, S. P., Roy, S., & Roy, S. (2012). Nicotine induced pro-oxidant and antioxidant imbalance in rat lymphocytes: in vivo dose and time dependent approaches. *Toxicology Mechanisms and Methods*, *22*(9), 711-720.

Erfani, M. N., Bahrami, M., Morovati, H., & Najafzadehvarzi, H. (2013). The protective effect of Aloe vera on histological and histometrical structure of diabetic rat testis. *Iranian Veterinary Journal*, *9*(2), 78-87.

Evtyugin, D. D., Magina, S., & Evtuguin, D. V. (2020). Recent advances in the production and applications of ellagic acid and its derivatives. A review. *Molecules*, *25*(12), 2745.

Golmei, P., Kasna, S., Roy, K. P., & Kumar, S. (2024). A review on pharmacological advancement of ellagic acid. *Journal of Pharmacology and Pharmacotherapeutics*, *15*(2), 93-104.

Guo, X., Wang, H., Wu, X., Chen, X., Chen, Y., Guo, J., ... & Ge, R. S. (2017). Nicotine affects rat Leydig cell function in vivo and vitro via down-regulating some key steroidogenic enzyme expressions. *Food and Chemical Toxicology*, *110*, 13-24.

Gupta, A., Singh, A. K., Kumar, R., Jamieson, S., Pandey, A. K., & Bishayee, A. (2021). Neuroprotective potential of ellagic acid: a critical review. *Advances in Nutrition*, *12*(4), 1211-1238.

Guvvala, P. R., Ravindra, J. P., Selvaraju, S., Arangasamy, A., & Venkata, K. M. (2019). Ellagic and ferulic acids protect arsenic-induced male reproductive toxicity via regulating Nfe2l2, Ppargc1a and StAR expressions in testis. *Toxicology*, *413*, 1-12.

Harlev, A., Agarwal, A., Gunes, S. O., Shetty, A., & du Plessis, S. S. (2015). Smoking and male infertility: an evidence-based review. *The world journal of men's health*, *33*(3), 143-160.

Hosseinzadeh, A., Mehrzadi, S., Siahpoosh, A., Basir, Z., Bahrami, N., & Goudarzi, M. (2021). The ameliorative effect of ellagic acid on di-(2-ethylhexyl) phthalate-induced testicular structural alterations, oxidative stress, inflammation and sperm damages in adult mice. *Reproductive Biology and Endocrinology*, *19*(1), 146.

Jalili, C., Salahshoor, M. R., Moradi, M. T., Ahookhash, M., Taghadosi, M., & Sohrabi, M. (2017). Expression changes of apoptotic genes in tissues from mice exposed to nicotine. *Asian Pacific journal of cancer prevention: APJCP*, *18*(1), 239.

Kalantari, A., Shahrooz, R., Ahmadi, A., & Malekinejad, H. (2014). Evaluation of sperm quality in phenylhydrazine-induced hemolytic anemia impacts on mice and protective role of crocin. *Iranian Veterinary Journal*, *10*(1), 77-84.

Khazaeel, K., Daaj, S. A. Z., Sadeghi, A., Tabandeh, M. R., & Basir, Z. (2022). Potential protective effect of quercetin on the male reproductive system against exposure of Wistar rats to crude oil vapor: Genetic, biochemical, and histopathological evidence. *Reproductive toxicology*, *113*, 10-17.

Kushwaha, S., & Jena, G. B. (2014). Effects of nicotine on the testicular toxicity of streptozotocin-induced diabetic rat: intervention of enalapril. *Human & experimental toxicology*, *33*(6), 609-622.

Li, Y., Zhang, J., Zhang, L., Hu, C., Zhou, L., Cheng, Y., & Liu, Q. (2025). Ellagic acid (EA) ameliorates Alzheimer's disease by reducing Aβ levels, oxidative stress and attenuating inflammation. *European Journal of Pharmacology*, *986*, 177099.

Liu, Y., Chen, C., Hao, Z., Shen, J., Tang, S., & Dai, C. (2024). Ellagic Acid Reduces Cadmium Exposure-Induced Apoptosis in HT22 Cells via Inhibiting Oxidative Stress and Mitochondrial Dysfunction and Activating Nrf2/HO-1 Pathway. *Antioxidants*, *13*(11), 1296.

Madi, N. M., Abo El Gheit, R. E., Barhoma, R. A., El Saadany, A., Alghazaly, G. M., Marea, K., & El-Saka, M. H. (2021). Beneficial impact of Nesfatin-1 on reproductive dysfunction induced by nicotine in male rats: Possible modulation of autophagy and pyroptosis signaling pathways. *Physiology International*, *108*(2), 185-201.

Mahmoud, A. A., Abdel-Aziz, H. O., Elbadr, M., & Elbadre, H. (2021). Effect of nicotine on STAT1 pathway and oxidative stress in rat lungs. *Reports of Biochemistry & Molecular Biology*, *10*(3), 429.

Marinucci, L., Balloni, S., Bellucci, C., Lilli, C., Stabile, A. M., Calvitti, M., ... & Arato, I. (2020). Effects of nicotine on porcine pre-pupertal sertoli cells: An in vitro study. *Toxicology In Vitro*, *67*, 104882.

Mehrzadi, S., Bahrami, N., Mehrabani, M., Motevalian, M., Mansouri, E., & Goudarzi, M. (2018). Ellagic acid: A promising protective remedy against testicular toxicity induced by arsenic. *Biomedicine & pharmacotherapy*, *103*, 1464-1472.

Mohammadghasemi, F., Khanaki, K., Moravati, H., & Faghani, M. (2021). The amelioration of nicotine-induced reproductive impairment in male mouse by Sambucus ebulus L. fruit extract. *Anatomy & Cell Biology*, *54*(2), 232-240.

Mosadegh, M., Hasanzadeh, S., & Razi, M. (2017). Nicotine-induced damages in testicular tissue of rats; evidences for bcl-2, p53 and caspase-3 expression. *Iranian Journal of Basic Medical Sciences*, *20*(2), 199.

Mosbah, R., Yousef, M. I., & Mantovani, A. (2015). Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: the protective effects of green tea extract. *Experimental and Toxicologic Pathology*, *67*(3), 253-259.

Naraki, K., Rameshrad, M., & Hosseinzadeh, H. (2022). Protective effects and therapeutic applications of ellagic acid against natural and synthetic toxicants: A review article. *Iranian journal of basic medical sciences*, *25*(12), 1402.

Ni, K., Spiess, A. N., Schuppe, H. C., & Steger, K. (2016). The impact of sperm protamine deficiency and sperm DNA damage on human male fertility: a systematic review and meta-analysis. *Andrology*, *4*(5), 789-799.

Omolaoye, T. S., El Shahawy, O., Skosana, B. T., Boillat, T., Loney, T., & Du Plessis, S. S. (2022). The mutagenic effect of tobacco smoke on male fertility. *Environmental Science and Pollution Research*, *29*(41), 62055-62066.

Oyeyipo, I. P., Raji, Y., & Bolarinwa, A. F. (2014). Antioxidant profile changes in reproductive tissues of rats treated with nicotine. *Journal of human reproductive sciences*, *7*(1), 41-46.

Oztekin, U., Caniklioglu, M., Firat, F., Atac, F., Doganyigit, Z., Gocmen, A. Y., ... & Tokpinar, A. (2020). Carob attenuates nicotine-induced oxidative stress and intratesticular damage in male rats. *Andrologia*, *52*(9), e13670.

Paccola, C. C., & Miraglia, S. M. (2016). Prenatal and lactation nicotine exposure affects Sertoli cell and gonadotropin levels in rats. *Reproduction*, *151*(2), 117-133.

Rahimi-Madiseh, M., Mohammadi, M., Hassanvand, A., Ahmadi, R., Shahmohammadi, M., & Rostamzadeh, A. (2020). Assessment of the toxicity effects of nicotine on sperm and IVF and the potential protective role of silymarin—an experimental study in mice. *Middle East Fertility Society Journal*, *25*(1), 14.

Ribas-Maynou, J., Yeste, M., Becerra-Tomás, N., Aston, K. I., James, E. R., & Salas-Huetos, A. (2021). Clinical implications of sperm DNA damage in IVF and ICSI: updated systematic review and meta-analysis. *Biological Reviews*, *96*(4), 1284-1300.

Rostami, A., Vakili, S., Koohpeyma, F., Jahromi, B. N., Aghajari, Z. A., Mahmoudikohani, F., ... & Noroozi, S. (2022). Ellagic acid effects on testis, sex hormones, oxidative stress, and apoptosis in the relative sterility rat model following busulfan administration. *BMC Complementary Medicine and Therapies*, *22*(1), 170.

Sadeghi, A., Farokhi, F., Shalizar-Jalali, A., & Najafi, G. (2020). Protective effect of vitamin E on sperm quality and in vitro fertilizing potential and testosterone concentration in polyvinyl chloride treated male rats. *Veterinary Research Forum*, *11*(3), 257.

Sadeghi, A., Ghahari, L., Yousefpour, M., Khazaeel, K., & Zareian, P. (2023). Inhalation exposure to crude oil vapor induces behavioural deficits by increasing oxidative stress and histopathological changes in rat hippocampus: Quercetin therapeutic approach. *Journal of Chemical Neuroanatomy*, 131, 102290.

Sansone, L., Milani, F., Fabrizi, R., Belli, M., Cristina, M., Zagà, V., Russo, P. (2023). Nicotine: from discovery to biological effects. *International Journal of Molecular Sciences*, *24*(19), 14570.

Ukwenya, V. O., Olawuyi, T. S., Adam, A. M., & Ukwenya, M. U. (2020). Hormonal changes and redox imbalance in nicotine-induced testicular toxicity: the mitigating influence of D-ribose l-cysteine. *The Journal of Basic and Applied Zoology*, *81*(1), 48.

Zangene, S., Morovvati, H., & Anbara, H. (2025). The effect of Polystyrene Microplastics on Histomorphometric Parameters of Testis in Male Mature Mice. *Iranian Veterinary Journal*, *20*(4), 62-73.

Zhang, W., Lin, H., Zou, M., Yuan, Q., Huang, Z., Pan, X., & Zhang, W. (2022). Nicotine in inflammatory diseases: anti-inflammatory and pro-inflammatory effects. *Frontiers in immunology*, *13*, 826889.