http://www.ijwhr.net
doi 10.15296/ijwhr.2016.03
Open Access Original Article

IJWHR
International Journal of Women's Health and Reproduction Sciences
Vol. 4, No. 1, January 2016, 8–12
ISSN 2330-4456

The Effect of Methamphetamine on Oocyte Quality, Fertilization Rate and Embryo Development in Mice

Maryam Nezhad Sistani[1], Marefat Ghaffari Novin[1,2], Fatemeh Fadaee Fathabadi[1*], Mohamad Salehi[2,3], Maryam Salimi[1], Zahra Shams Mofarahe[1], Mahdi Hadi[4]

Abstract

Objectives: Methamphetamine (METH) is an illicit psychoactive drug. There are different reasons of abusing METH such as for recreational use and sexual satisfaction. This study was designed to investigate the effects of short and long-term use of METH on oocyte and embryo development in mice.

Materials and Methods: In this study, 75 female NMRI mice were divided into five groups of 15. The groups consisted of a control group, experimental groups A and B, which received METH (10 mg/kg/day) intraperitoneally for 2 and 14 consecutive days, respectively and two sham groups A and B, which only received saline. After the last injection, 10 international units pregnant mare serum gonadotropin (IU PMSG) and 10 IU human chorionic gonadotropin (HCG) were administered intraperitoneally for induction of ovulation. Then, all the mice were sacrificed to aspirate their oocytes for further evaluation. In vitro fertilization (IVF) was done by using mature oocytes and embryo development was investigated up to the blastocyst stage. Data was analyzed using SPSS and non-parametric Mann-Whitney test.

Results: The number of fragmented oocytes in experimental group A was significantly increased in comparison with experimental group B and control group ($P<0.05$). Also there were significant differences in the number of unfertilized oocytes and early cleavage stage embryos in experimental group B in comparison with other groups that indicates the reduction of fertilization rate in experimental group B ($P<0.05$).

Conclusion: Short-term administration of METH in mice can affect oocyte quality but had no effect on early embryo development, while the long-term administration may affect oocyte and embryo development in early cleavage stage.

Keywords: Cleavage, Embryonic development, Fertilization in vitro, Methamphetamine, Oocytes, Preimplantation

Introduction

Methamphetamine (METH) is an illicit psychostimulant drug belonging to amphetamine category. Comparing amphetamine derivatives, METH is stronger and has a more permanent effect on central nervous system in human brain (1). Easy preparation and low price of METH has increased its abuse (2), such that today, we have encountered the unpleasant social consequences of drug abuse in Iran which does not subside (3). There are different reasons of abusing METH such as increasing the strength to bear a long period of a heavy labor (4), for fun and sexual intimacy and also losing weight. It seems that the main users of METH in Iran, abuse it to have fun and develop sexual satisfaction (3).

The use of METH among women is significantly associated with an increase in high risk sexual behaviors leading to increased sexual transmission of diseases such as HIV/AIDS and increased abortion rate (5,6). Recently, the adverse effects of METH on the reproduction system has got attention as METH is considered to be a teratogenic and embryotoxic drug (7). Various studies have been done studying the effect of METH on testis tissue and spermatogenesis process, which showed the reduction of spermatogenesis process (8) and also induction of apoptosis in seminiferous tubules in rat testis (9). In another research, the effect of ecstasy, as one of the amphetamine derivatives, on oocyte quality and fertilization rate was studied and the results showed the probable effect of this drug on the oocyte quality and fertilization rate (10).

This study was designed to evaluate the effect of short and long-term METH administration on oocyte quality, fertilization rate and the quality of embryo in pre-implantation stage in mice.

Materials and Methods

All reagents and materials were purchased from Sigma chemical Corporation (St. Louis Mo, USA) except where mentioned otherwise. Also T6 culture medium protocol was obtained from manipulating the Mouse Embryo book.

Received 5 March 2015, **Accepted** 12 October 2015, **Available online** 1 January 2016

[1]Department of Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [2]Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Department of Biotechnology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Department of Embryology at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

***Corresponding author:** Fatemeh Fadaee Fathabadi, Department of Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +9821-22439976, Email: fadayi@sbmu.com

Animals

A total of 75 female NMRI mice were used as experimental subjects. All mice were obtained from the Pasteur Institute, Iran. The animals were housed in a room for 2 weeks under a 12 hours light/dark cycle with easy access to water and food. Temperature and humidity in the animal colony were monitored daily. The animals (n = 75) were divided randomly into 5 groups (with 15 mice each). Control group, experimental group A (received 10 mg/kg METH for 2 successive days), Experimental group B (received 10 mg/kg METH for 14 successive days). Sham group A and B also received physiological saline intraperitoneally (IP) corresponding to the experimental groups A and B.

Preparation of Methamphetamine

METH was obtained from Drug Protection Administration, Iran and after being analyzed in School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran, the purity of 98% was determined. After dilution of the material in physiological saline, it was prepared for intraperitoneal injection.

In Vitro Fertilization Process

Oocyte Collection

Five hours after the last dose of METH, all the mice in experimental groups A and B and other groups were superovulated with intraperitoneal administration of 10 IU PMSG (Folligon 1000 for Animal Use-Intervet Company, Canada) followed by 10 IU of human chorionic gonadotropin (HCG) (Pregnil 500, Darupakhsh, Iran) 48 hours later. The mice were sacrificed 13 or 14 hours after injection of HCG to bring out oviducts of both sides. Then the oviducts were transferred in T6 medium without bovine serum albumin (BSA) and dissected with two insulin syringes under stereomicroscope. The oocytes cumulus complexes were collected from dissect medium by Pasteur pipette and placed in T6 fertilization medium containing 15% BSA. Until sperm preparation oocytes cumulus complexes were incubated at 37°C and 6% CO2.

Sperm Suspension Preparation

For every three females, one male mouse was sacrificed and cauda epididymis of both sides were excised and chopped using a pair of small scissors, then placed in 1 ml T6 medium in a test tube and incubated at 37°C and 6% CO2 for 1 hour. After swim-up period, 5×106 sperms were added to 50 µl of T6 medium droplets containing 10 oocytes and incubated at 37°C under 6% CO2 in humidified air for 4-6 hours. Then oocytes were transferred to 30 µl of culture medium (T6 medium containing 4% BSA) droplets and incubated again.

The Assessment of Embryo Development

After in vitro fertilization (IVF) process and observing the pronucleus (PN), embryo development was assessed on days 1, 2, 3, 4 and 5 after transferring the inseminated oocytes in culture medium under Nikon SMZ800 stereomicroscope (Nikon Corporation, Tokyo, Japan). The percentage of arrested embryo was determined.

Statistical Analysis

All statistical analysis was performed using SPSS 19 software. The obtained data was expressed as means ± SD. The means of fragmented oocytes, fertilized oocytes, 2-cells, 4-cells, 8-cells embryos, morula and blastocyst were compared by non-parametric Mann-Whitney test between the groups. Statistically significant difference was accepted at $P < 0.05$.

Results

As illustrated in Table 1, some of oocytes were fragmented in feature. The results showed that in the experimental group A, the percentage of fragmented oocytes was statistically higher than the other studied groups. So there were statistically differences between experimental A group with control and sham A groups. In this respect, there was statistical difference between experimental groups A and B.

Furthermore unfragmented oocytes were transferred to IVF environment to survey fertilization rate in studied groups. Statistical analysis showed the percentage of unfertilized oocytes in the control group and experimental groups A and B were 17.3%, 24.3% and 43.2%, respectively. There were statistical differences between experimental group B and the two other groups. So this result was obtained between experimental B and sham B groups ($P < 0.05$).

The percentage of embryos at different developmental stages in studied groups is summarized in Table 2. Some of unfragmented oocytes were fertilized and developed to

Table 1. The Percent of Fragmented and Unfragmented Oocytes (Mean % ± SEM)

	Control	Sham A	Experimental A	Sham B	Experimental B
Total oocytes	198	186	189	213	202
Fragments oocyte	12 (7.28 ± 2.45)[a]	14 (8.89 ± 2.53)[b]	43 (26.02 ± 8.15)[a,b,c]	17 (6.26 ± 2.39)	18 (6.92 ± 2.55)[c]
Un-fragmented oocyte	186 (92.71 ± 2.45)[d]	172 (91.1 ± 2.53)[e]	146 (74 ± 10.16)[d,e,f]	196 (93.73 ± 3.39)	184 (93.08 ± 2.55)[f]

Control (never received substance), Sham A (10 mg/kg/day saline for 2 days), Experimental A (10 mg/kg/day METH for 2 days), Sham B (10 mg/kg/day saline for 14 days), Experimental B (10 mg/kg/day METH for 14 days). According this table, the number of fragmented oocyte was significantly increased in experimental A group in comparison with other groups. Within the same column, values with same letters were significantly different ($P < 0.05$).

Note: Identical letters among the groups in the table represent significant relationship between them.

Table 2. The Percentage of Embryo Developmental Stages in Studied Groups (Mean % ± SEM)

Group	Control	Sham A	Experimental A	Sham B	Experimental B
2-cell stage	157 (82.66±5.24)[a]	129 (73.7±2.05)	106 (75.7 ±4)[b]	157 (78.05±7.67)[c]	109 (56.79±2)[a,b,c]
4-cells stage	140 (75.15±9.03)[d]	107 (60.54±4.35)	104 (57.6± 4.26)[e]	153 (66.01±5.80)[f]	92 (40.40±4.08)[d,e,f]
8-cells stage	123 (61.65±11.20)[g]	87 (47.3±5.6)	57 (43.73±6.28)	107 (51.1±5.8)	65 (29.47±5.06)[g]
Morula	60 (31.31±4.59)	77 (41.58±5.15)	45 (37.40±8.36)	61 (38.85±5.33)	48 (26.79±4.82)
Blastocyst	49 (26.38±2.64)	66 (30.7±4.05)	26 (22.58±5.84)	38 (16.9±7.2)	23 (12.64±5.63)

Control (never received substance), Sham A (10 mg/kg/day saline for 2 days), Experimental A(10 mg/kg/day METH for 2 days), Sham B (10 mg/kg/day saline for 14 days), Experimental B (10 mg/kg/day METH for 14 days). This table shows the reduction of cleavage rate in experimental group B in comparison with other groups. Within the same column, values with same letters were significantly different ($P < 0.05$).
Note: Identical letters among the groups in the table represent significant relationship between them.

2-cells stage embryos and even to blastocyst stage, whereas some of them arrested in different developmental stages. At least 2-cells stage embryo formation was observed in experimental group B. Also our results showed that there was no statistical difference in the percentage of embryo at different stages between the experimental group A and control group ($P > 0.05$).

As shown in Figures 1 and 2, There were statistical differences between experimental group B and the two other groups. So this result was obtained between experimental B and sham B groups ($P < 0.05$).

Discussion

According to the spread of METH abuse among young women, this study was designed to evaluate the effects of METH on oocyte quality, fertilization rate and the development of embryos in pre-implantation stage.

The results demonstrated statistical relation between METH abuse and poor quality oocyte, low fertilization rate and poor quality embryos.

Our results showed that short-term abusing of METH may lead to increasing number of fragmented oocytes and decrease in good quality oocyte. In agreement with our results, Haji-Maghsoodi et al reported that administration of short-term ecstasy in mice can affect both oocytes quality and fertilization potential of oocytes (10).

According to previous studies, METH can affect neural cells (11) and T lymphocytes (12) and cause fragmentation of mitochondria via increasing ROS production. Therefore, it seems that fragmented oocyte feature in this study may be due to fragmentation of mitochondria. On the other hand some studies reported that nuclear and cytoplasmic maturation of oocytes are necessary for fertilization and embryo development (13). Oocyte nuclear maturation is determined by releasing of first polar body (14) and oocyte cytoplasmic maturity is estimated by mitochondrial distribution (15). So METH may impair cytoplasmic maturity of oocytes by damaging the mitochondria.

Meanwhile, METH can induce onset of apoptosis process by production of apoptosis related proteins via increasing the expression of P53 as a transcription factor (16). It can also diffuse into the mitochondria and reduce ATP synthesis (17), increase the apoptotic proteins including Bad, Bax and Bid and decrease anti-apoptotic proteins including Bcl2 and Bcl-xl (18,19). In apoptosis process some alternation are seen in both nucleus and cytoplasm of cells. In the nucleus, apoptosis process causes chromatin condensation and fragmentation and finally creates an abnormal morphological nucleus. Also cytoplasm changes may occur as shrinkage of cells without rupture of cell membrane and create the fragmented appearance of cells (20).

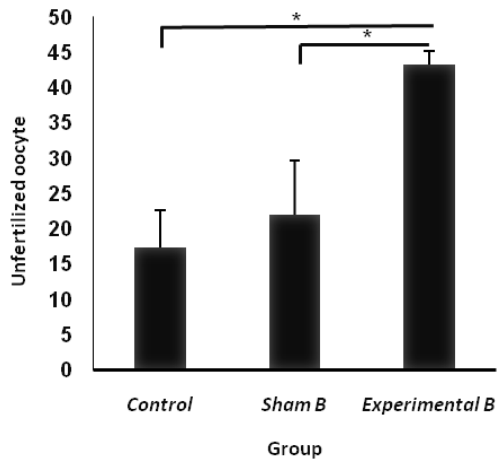

Figure 1. In unfertilized oocyte there were statistical differences between experimental group B with control and sham B groups, so the percentage of unfertilized oocyte was increased in Experimental group B ($P < 0.05$).

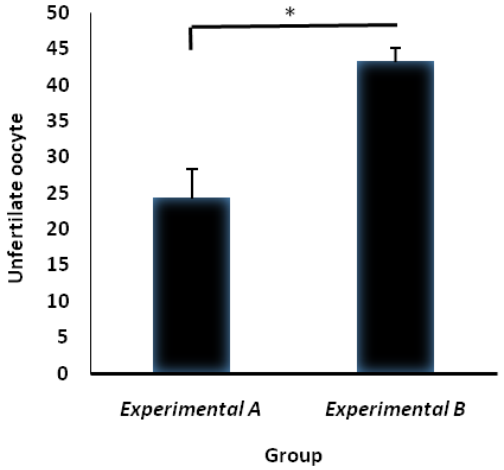

Figure 2. In unfertilized oocytes, in comparison between two experimental groups there were statistical differences. Figure shows that the percentage of unfertilized oocyte was increased in Experimental group B ($P < 0.05$).

Moreover, some studies reported that the administration of METH increased the P53 expression, caspase activity and Bax/Bcl2 in rat testis and it could change the number and quality of sperms (21). Therefore, it seems that the induction of apoptosis process is another reason for creating of fragmented oocyte feature.

The results demonstrated that long-term abuse of METH can decrease fertilization rate. It seems that METH can affect the accumulation of ROS in oocytes and subsequently decrease fertilization rate. The ROS can damage cell content such as lipids, proteins, DNA and mitochondria via the rapid diffusion of cells membrane (22).

Mitochondria are essential organelles in cytoplasm of somatic cells and oocytes. It can produce the required oocyte ATP for fertilization (23,24). Therefore, there is relation between the number of oocyte mitochondria and fertilization rate (25). Unlike somatic cells, the oocyte mitochondria have only one gene copy (26,27). Mitochondrial DNA of oocytes is more exposed to mutation because of lacking repair system in comparing with other cells (28). This matter will decrease fertilization rate in oocytes which are exposed to long term administration of METH.

Our finding also showed that long-term abuse of METH can affect the early cleavage stage of development and stop it.

This finding is compatible with some studies. Kaufmann et al (29) and Kim et al (30) investigated the effects of cocaine on mouse embryo development. They showed that the potential of embryo development until blastocyst stage was reduced in comparison with control group (29,30). It seems that METH such as Cocaine has toxic effects on reproductive system and as a small lipophilic molecule, it can penetrate into the ovaries like cocaine and interfere in the DNA synthesis, cellular proliferation and cell cytoskeleton function and even affect the level of genes expression. Because mother genome is activated in early cleavage stage (31), as a result, insufficient material for cleavage can be transmitted to embryo by poor quality oocytes and cause cleavage stopping (31).

There is a possibility that METH can damage DNA and RNA (32,33) of oocytes via increasing ROS and consequently it can be involved in the arrest of embryos derived from oocytes with poor quality.

Conclusion

Short-term abuse of METH may affect the quality of the oocytes, but does not affect early embryo development stages, while the long-term abuse of METH may also reduce the rate of fertilization and embryo development in the early stages of cleavage.

Further studies on the effect of METH on the cellular and molecular operation of ovarian tissue and oocyte maturation are recommended.

Ethical issues

This study was approved by the Ethics Committee of the Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.REC.1392.337).

Conflict of interests

All the authors have reported no conflict of interest.

Financial support

This study was sponsored via Shahid Beheshti University of Medical Sciences and Cellular and Molecular Biology Research Center.

Acknowledgments

None to be declared.

References

1. Lake CR, Quirk RS. CNS stimulants and the look-alike drugs. Psychiatr Clin North Am. 1984;7(4):689-701.
2. Kulsudjarit K. Drug problem in southeast and southwest Asia. Ann N Y Acad Sci. 2004;1025(1):446-457. doi:10.1196/annals.1316.055.
3. Zarghami M. Methamphetamine has changed the profile of patients utilizing psychiatric emergency services in Iran. Iranian Journal of Psychiatry and Behavioral Sciences. 2011;5(1):1-5.
4. Sadock BJ, Sadock VA, Sussman N. Kaplan and Sadock's pocket handbook of psychiatric drug treatment. USA: Lippincott Williams & Wilkins; 2005.
5. Holder MK, Hadjimarkou MM, Zup SL, et al. Methamphetamine facilitates female sexual behavior and enhances neuronal activation in the medial amygdala and ventromedial nucleus of the hypothalamus. Psychoneuroendocrinology. 2010;35(2):197-208. doi:10.1016/j.psyneuen.2009.06.005.
6. Holder MK, Veichweg SS, Mong JA. Methamphetamine-enhanced female sexual motivation is dependent on dopamine and progesterone signaling in the medial amygdala. Horm Behav. 2015;67:1-11. doi:10.1016/j.yhbeh.2014.10.004
7. Yamamoto Y, Yamamoto K, Abiru H, Fukui Y, Shiota K. Effects of methamphetamine on rat embryos cultured in vitro. Biol Neonat. 1995;68(1):33-38. doi:10.1159/000244215.
8. Heidari-Rarani M, Noori A, Ghodousi A. Effects of methamphetamine on pituitary gonadal axis and spermatogenesis in mature male rats. Zahedan J Res Med Sci. 2014;16(12):35-40. [Persian].
9. Nudmamud-Thanoi S, Thanoi S. Methamphetamine induces abnormal sperm morphology, low sperm concentration and apoptosis in the testis of male rats. Andrologia. 2011;43(4):278-282. doi: 10.1111/j.1439-0272.2010.01071.x.
10. Haji-Maghsoudi F, Khalili MA, Karimzade A. Effects of MDMA (ecstasy) on oocyte quality and fertilization Rate in Mice. J Reprod Infertil. 2010;11(2):77-85. [Persian]
11. Brown JM, Yamamoto BK. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. Pharmacology & therapeutics.

2003;99(1):45-53. doi:10.1016/S0163-7258(03)00052-4.
12. Potula R, Hawkins BJ, Cenna JM, et al. Methamphetamine causes mitrochondrial oxidative damage in human T lymphocytes leading to functional impairment. J Immunol. 2010;185(5):2867-2876. doi: 10.4049/jimmunol.0903691.
13. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reprod Fertil Dev. 1996;8(4):48548-9. doi:10.1071/RD9960485.
14. Fulka J, First N, Moor R. Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol Hum Reprod. 1998;4(1):41-49. doi:10.1093/molehr/4.1.41.
15. Torner H, Brüssow KP, Alm H, Ratky J, Pöhland R, Tuchscherer A. Mitochondrial aggregation patterns and activity in porcine oocytes and apoptosis in surrounding cumulus cells depends on the stage of pre-ovulatory maturation. Theriogenology. 2004;61(9):1675-1689. doi:10.1016/j.theriogenology.2003.09.013
16. Imam SZ, Itzhak Y, Cadet JL, Islam F, Slikker W, Ali SF. Methamphetamine-induced alteration in striatal p53 and bcl-2 expressions in mice. Mol Brain Res. 2001;91(1-2):174-178. doi:10.1016/S0169-328X(01)00139-5.
17. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu Rev Genet. 2005;39:359. doi:10.1146/annurev.genet.39.110304.095751.
18. Deng X, Cadet JL. Methamphetamine-induced apoptosis is attenuated in the striata of copper–zinc superoxide dismutase transgenic mice. Mol Brain Res. 2000;83(1-2):121-124. doi:10.1016/S0169-328X(00)00169-8.
19. Jayanthi S, Deng X, Bordelon M, Mccoy MT, Cadet JL. Methamphetamine causes differential regulation of pro-death and anti-death Bcl-2 genes in the mouse neocortex. FASEB J. 2001;15(10):1745-1752. doi: 10.1096/fj.01-0025com.
20. Jurisicova A, Varmuza S, Casper R. Programmed cell death and human embryo fragmentation. Mol Hum Reprod. 1996;2(2):93-8. doi:10.1093/molehr/2.2.93.
21. Lin JF, Lin YH, Liao PC, et al. Induction of testicular damage by daily methamphetamine administration in rats. Chinese J Physiol. 2014;57(1):19-30. doi: 10.4077/CJP.2014.BAB155.
22. Wells PG, Bhuller Y, Chen CS, et al. Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. Toxicol Appl Pharmacol. 2005;207(2):354-366. doi:10.1016/j.taap.2005.01.061
23. Montoya J, López-Pérez MJ, Ruiz-Pesini E. Mitochondrial DNA transcription and diseases: past, present and future. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2006;1757(9-10):1179-1189. doi:10.1016/j.bbabio.2006.03.023
24. Novin MG, Noruzinia M, Allahveisi A, et al. Comparison of mitochondrial-related transcriptional levels of TFAM, NRF1 and MT-CO1 genes in single human oocytes at various stages of the oocyte maturation. Iran Biomed J. 2015;19(1):23. doi:10.6091/ibj.1400.2015.
25. Santos TA, El Shourbagy S, John JC. Mitochondrial content reflects oocyte variability and fertilization outcome. Fertil Steril. 2006;85(3):584-591. doi:10.1016/j.fertnstert.2005.09.017.
26. Pikó L, Matsumoto L. Number of mitochondria and some properties of mitochondrial DNA in the mouse egg. Dev Biol. 1976;49(1):1-10. doi:10.1016/0012-1606(76)90253-0.
27. Pikó L, Taylor KD. Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. Dev Biol. 1987;123(2):364-374. doi:10.1016/0012-1606(87)90395-2.
28. Catt JW, Henman M. Toxic effects of oxygen on human embryo development. Hum Reprod. 2000;15(suppl 2):199-206. doi:10.1093/humrep/15.suppl_2.199.
29. Kaufmann RA, Armant DR. In vitro exposure of preimplantation mouse embryos to cocaine and benzoylecgonine inhibits subsequent development. Teratology. 1992;46(1):85-89. doi:10.1002/tera.1420460112.
30. Kim SH, Yang BK, Kim HC, Jhoo WK. Effect of cocaine administration on the development of mouse embryos. Arch Pharma Res. 1994;17(4):209-212.
31. Van Blerkom J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. Mitochondrion. 2011;11(5):797-813. doi:10.1016/j.mito.2010.09.012.
32. Li Y, Trush MA. DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu (II)/Cu (I) redox cycle and reactive oxygen generation. Carcinogenesis. 1993;14(7):1303-1311. doi: 10.1093/carcin/14.7.1303.
33. Barenys M, Macia N, Camps L, et al. Chronic exposure to MDMA (ecstasy) increases DNA damage in sperm and alters testes histopathology in male rats. Toxicol Lett. 2009;191(1):40-46. doi:10.1016/j.toxlet.2009.08.002.