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Original Article

Attenuation of Detrimental Effects of Formaldehyde on Sperm Chromatin Quality and Rate of Apoptosis in Mice by Curcumin

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ABSTRACT

Background: The present study aimed to evaluate the sperm chromatin quality and rate of apoptosis in formaldehyde (FA)-exposed mice and the possible protective effects of curcumin (Cur) on the fertility potential of spermatozoa.

Methods: Twenty-four adult male NMRI mice were randomly divided into three groups: Group I (control), group II (Sham) received Cur solvent (2 ml/day, intraperitoneal), group III received FA (10 mg/kg, intraperitoneal), and group IV received FA+Cur (100 mg/kg, intraperitoneal). After 35 days, the spermatozoa from right cauda epididymis were analyzed using Chromomycin A3 (CMA3) and TUNEL staining.

Result: Regarding CMA3 and TUNEL tests, the data revealed a significant increase (P<0.05) in control and sham groups compared to the FA and FA+Cur groups. There was also a significant decrease in CMA3 tests and apoptosis in FA+Cur group compared to FA group (P<0.05).

Conclusion: Cur, as a potent antioxidant, can attenuate detrimental effects of FA on the chromatin quality and apoptosis in an experimental animal model.

Keywords: Formaldehyde, Curcumin, Chromatin quality, Apoptosis, Sperm, Mice

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Introduction

It is important to understand that the quality of sperm in humans and other animals has declined in the last 50 years (1) and this is due to considerable changes in human physical, chemical, and biological environments (2, 3). Recent studies have reported the physical and chemical hazards of the jobs on sperm quality (4). Formaldehyde (FA) (CH_2O) is a family member of aldehydes with the simplest organic molecule, colorless, flammable element, and stimulant with spicy odor that plays an important role in the global economy and is considered as an environmental pollutant. FA is widely used in cosmetic and work environments including beauty salons, hospitals, industries, room outlines to fix the body, and histology and histopathology techniques (5, 6). Daily use and occupational contact with FA was estimated to be more than PPm3 (7) whereas its inner concentration in rats, monkeys, and humans is 0.1 mM (8, 9). Several studies have reported harmful effects of FA on reproductive, breathing, and hematological systems (5, 10, 11). It has been shown that FA leads to testicular atrophy and reduction of testicular weight, the diameter of the semen tubes, the height of the epithelium of semen tubes, the movement and the number of spreads (5, 6, 12). Also, FA can inhibit spermatogenesis and cause the induction of apoptosis in testicular epithelial cells (10, 13-15). FA is a genotoxic factor which can cause damage to DNA. It is also a carcinogen, causing mutations and chromosomal anomalies in cytogenic processes (8). It is well known that the production of free reactive oxygen species (ROS) or inadequate deposits during spermatogenesis can cause irreversible DNA gamete (16) as well as apoptosis during maturation of sperm (17). Protamines, which are proteins located within the sperm DNA molecule, have an important role in the dense DNA molecule for having high arginine (18). It has been shown that the density of DNA molecules is 6 times higher than that of mitotic, which are presented as smaller circles compared with somatic cells and are connected to the core matrix. Protamines cause the condensation of these rings and their stability through the disulfide bands (19, 20). If these bands are broken, the DNA rings are opened and the aura around the structures located in the center of the nucleus (17). As sperm can have fertility, it should be able to be decondensed at the right time during the fertilization process (21). It has been shown that disruption of the genome within the sperm nucleus has a negative relationship with sperm fertility ability in vitro and in-vivo (22). In humans, there is a balance between ROS production and the antioxidant system of sperm in the male genital tract (23). FA by increasing the production of ROS in many tissues causes induction of cell death and oxidative damage (10). ROS can directly and indirectly cause DNA damage to the sperm by activating caspases and sperm (24). Small amounts of ROS are necessary for sperm activities such as capacity building and acrosomal reaction (25). But excessive ROS production has a negative relationship with sperm function and its morphology (26). Several studies have been conducted on the use of different antioxidants such as curcumin (Cur) in order to prevent oxidative damage to the testes (27-32). Cur is a phenolic compound that is extracted from the roots of the Corcuma longa, a plant which is known as turmeric and has a range of pharmacological activities including antioxidant, anti-inflammatory, anticancer, and antimicrobial effects (33). In Asian medicine, Cur has been used for long periods of time because of its medicinal properties (34). Cur is capable of modulating the prescription factors, receptors, kinases, cytokines, enzymes, and growth factors involved in many biochemical and molecular activities (35). The protective effect of Cur on the testes following the use of lindane (36), alcohol (37), cisplatin (38), aflatoxin (39), sodium arsenite (40), and artesunate (41) has been reported. As the protective effect of Cur on testicular tissue, and sperm parameters has been reported, this study was conducted to evaluate its effects on chromatin quality and sperm DNA structure against the induction damage to the testes by FA.

Materials and Methods

In this experimental study, 24 male NMRI mice; weighing 30-35 gr and aged 6-8 weeks were obtained from animal house of Yazd Reproductive Science Institute. The mice were kept under a 12:12 hr light/dark cycle at a temperature of 22-25°C and were fed with standard animal chow and water. The animals were divided into four groups (n = 6). Group I was control (no injection), Group II (Sham) received curcumin solvent (DMSO, 2 ml/day), Group III received formaldehyde (10 mg/kg, intraperitoneal) (42), and Group IV received formaldehyde (10 mg/kg) + curcumin (100

mg/kg), intraperitoneal (43, 44). Cur was dissolved with dimethyl sulfoxide (DMSO).

All groups were treated in mouse spermatozoa period (35 days), and after the treatment period, they were killed by the displacement of the neck vertebrae. The dissected epididymis of each animal was transferred into 1 mL pre-warmed Ham's F10 medium and cut to small slices to swim out the sperm cells into the medium. The samples were incubated at 37°C for 30 min.

Sperm DNA evaluation

Sperm DNA integrity was evaluated using standard cytotoxic techniques including TUNEL and CMA3 (45, 46). All dyes and chemicals were purchased from Sigma Aldrich Company (St Louis, MO, USA).

Chromomycin A3 (CMA3) staining

The fluorescent dye of CMA3 is competing with protamine for binding to minor grooves of DNA which have CG-rich areas, and therefore, indirectly shows the amount of protamine deficiency in the chromatin structure. Sperm with protamine deficiency were colored using 100 ml solution of CMA3 and they were observed bright yellow with fluorescence microscopy and were referred to as sperm with immature chromatin (18).

To perform this test, after obtaining smear from each animal sample and drying in air, the slides were fixed in a refrigerator for 10 min by fixative Carnoy's solution, and then, were stained with 100 µl solution of CMA3 (Sigma-USA) for 10 min. In each slide, at least 200 spermatozoa were counted under fluorescent microscope and an appropriate filter of 470-460 nm with a magnification of X1000 was used (19). The number of sperm with brilliant yellow color (CMA3+) and sperm without luminosity (CMA3−) was determined.

TUNEL assay

In this method, the degree of integration of Deoxyuridine triphosphate (duTP) was analyzed into single- and double-stranded DNA during TDT-catalyzed reaction by fluorescence microscopy or by more accurate flow cytometry. Increasing this integration will increase the amount of damage to DNA. The TUNEL technique is also used to examine the apoptotic process, and the sperm that enter the process are also characterized by having fragmented DNA fragments (20).

After drying the expansions at room temperature, the slides were placed in absolute methanol for 4 min, and then, placed in PBS (×1) for 30 min. Subsequently, the expansions were incubated in a blocking solution (3% H_2O_2 in methanol) for 10 min at 15 to 25°C. After washing with PBS for 5 min, the expansions were incubated with 0.1% sodium terephthalate 0.1% sodium tetrachloride solution for 2 min at 2 to 8°C. The spread was then washed with PBS for 5 min. For each slurry, 5 µl of enzyme solution and 45 µl of labeled solution were mixed into a microtipo and added to all parts of the slide for 1 hr at 37°C in a dark and humid chamber. They were washed with PBS (3 times 5 min) and mounted with PBS (21). Next, they were observed by fluorescence microscope with a magnification of X1000. The number of spermatozoa with bright green nucleus (apoptotic sperm) and green nucleus without brightness (normal sperm) was determined.

Statistical analysis

Finally, the data of the groups were compared using ANOVA and Tukey's tests by SPSS version 19.0. Statistically significant level was considered at $P < 0.05$.

Results

DNA integrity outcome

CMA3

There was a significant difference in the percentage of sperm with protamine defect between control group and FA and FA+Cur groups ($P = 0.000$) (Figure 1).

A significant defect in the percentage of protamine defect was observed between FA and FA+Cur ($P = 0.000$) groups but there was no significant difference between the control and sham groups (Figure 1).

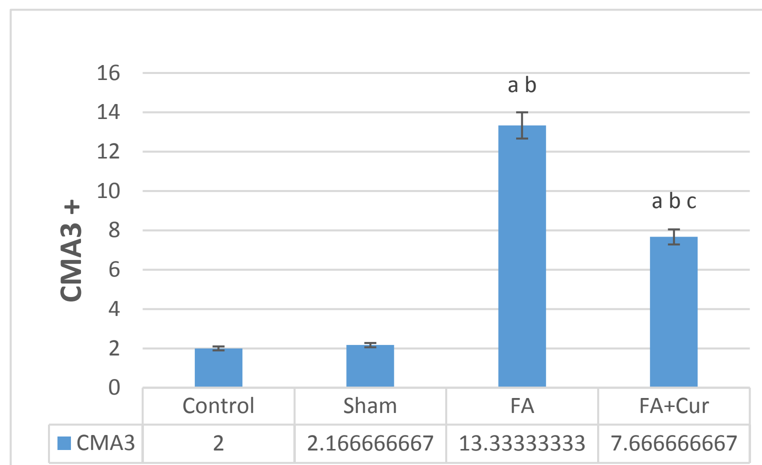

Figure 1. Results related to CMA3 assay in control, sham, FA, and FA+Cur groups.
a: Significant compared to control group.
b: Significant compared to sham group.
c: Significant compared to FA group ($P \leq 0.05$).

TUNEL

There was a significant difference in the mean number of DNA integrity between the control group and FA and FA+Cur groups (P = 0.000, P = 0.001) (Figure 2).

A significant defect increase in DNA integrity was observed between FA and FA+Cur groups (P = 0.023) but there was no significant difference between the control and sham groups (Figure 2).

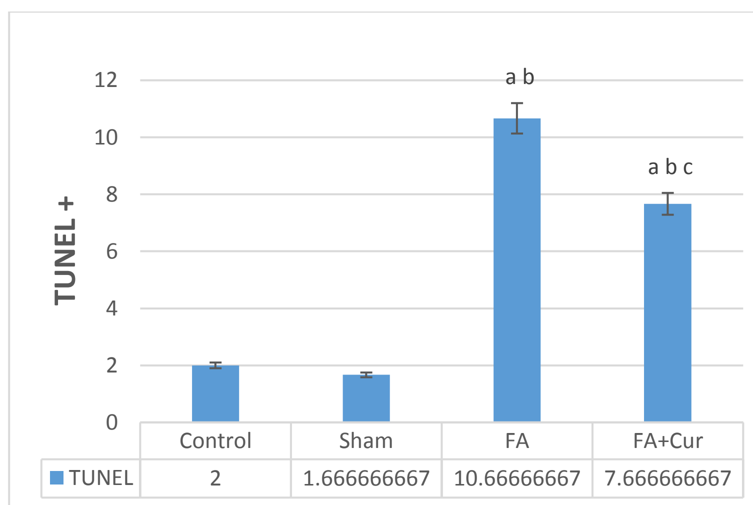

Figure 2. Results related to TUNEL test in control, sham, FA, and FA+Cur groups.
a: Significant compared to control group.
b: Significant compared to sham group.
c: Significant compared to FA group ($P \leq 0.05$).

Discussion

In the present experiment, curcumin, as an antioxidant, reduced FA-induced damage to sperm DNA structure. According to previous similar studies, FA negatively affects testicular structure, and consequently, sperm production and sperm parameters by free radical production. Oxygen is required for the functioning of organs, and its metabolites, such as ROS, can negatively affect either organs function, life or both of them (47). Zhou et al. (2011) reported that the structure and function of the rats exposed to 5.0 mg/m^3 FA was not significantly different from the control group but the quality and quantity of sperm, testis, seminiferous tubules diameter, action of superoxide dismutase and glutathione oxidase in rats exposed to 46.2 mg/m^3 FA decreased significantly compared to the control

group (48). Wang et al. (2012) reported an increase in the risk of prolonged pregnancy and spontaneous abortion in women whose husbands had an occupational contact with FA (49). Duong et al. (2011) showed that FA causes deficits in gametogenesis during spermatogenesis in rats (50). Betancourt-Martínez et al. (2015) observed that the percentage of DNA fragmentation in groups that were exposed to different doses of FA in Wistar rats were higher than control groups. They also showed that the percentage of sperm with fragmented DNA increased following the exposure to 5, 10, and 30 mg FA/kg body weight (51).

Previous studies have shown that antioxidants, including curcumin, can reduce the damage caused by FA. Cur is the main ingredient in turmeric, which is obtained from turmeric root (52). The results of this study showed that the amount of sperm protamine deficiency and DNA integrity in Cur group were lower than FA group, which indicates the protective effect of Cur in DNA structure. Desai et al. (2015) showed Cur improves the toxic effects of artensate on the testes of mice. Furthermore, administration of Cur at the dose level of 80 mg/kg bwt along with both doses of artesunate attenuated the adverse effects in male mice (41). Rashid et al. (2015) observed that the injection of 100 mg/kg of Cur for 8 weeks in diabetic rats have a protective effect against cellular damage to testicular cells. Cur is able to protect the testes against oxidative stress and endoplasmic reticulum by reducing blood sugar and testicular degradation markers, regulating the intracellular redox balance, reducing the inflammation caused by NF-kB activity, and activating the signaling mechanism related to PI3K/AKt. Furthermore, they suggested that this molecule is used as a potential treatment to prevent testicular functional disorders caused by diabetes-induced stress (53), which is consistent with the results of the present study. Noorafshan et al. (2010) observed that Cur can protect spermatocytes in metronidazole-treated mice, but it is not able to maintain rounded and drawn spermatides, testicular weight, and volume of germinal epithelium in the mice treated with metronidazole (54). Zha et al. (2018) showed that Cur may have therapeutic value in the treatment of diabetes-induced testicular injury due to its prevention of testicular apoptosis and attenuation of oxidative stress (55).

Ilbey et al. (2009) observed that Cur with ciptalsin prevented the damage to testis induced by this compound. However, CMN also may decrease the efficacy of chemotherapy based on CIS (38). Karimi et al. (2019) found that titanium oxide nanoparticles significantly reduced testicular weight, testosterone concentration, morphometric parameters, Johnsen's scoring, and sperm quality ($P < 0.01$), as well as a significant increase in histological criteria. Cur was found to have a potent protective effect against spermatogenesis defects induced by nanoparticles in mice, which is consistent with the results of the present study (56). Mahmoudi et al. (2017) observed that daily gavage of 100 mg/kg/day Cur could prevent structural impairments of testicles in the rats induced by Na-MBS (7 and 70 mg/kg/day) (57). Cheraghi et al. (2017) found that injection of 10 mg/kg curcumin in 40 male Wistar rats reduces the toxic effects of aluminum and improves the antioxidant status and sperm quality in male rats (58), which is consistent with the results of this study.

Conclusion

The results of this study showed that FA can damage sperm DNA and decrease its quality, which was shown with standard cytotoxic techniques. Cur as a useful and powerful antioxidant decreased this damage and improved sperm quality. However, further studies are needed to investigate the exact mechanism of curcumin's function in future.

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Ethical issues

The experimental project was confirmed by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Ethical code: IR.SSU.MEDICINE.REC.1396.103). Protocols of the Institutional Animal Care and Use Committee were followed during handling, maintenance, treatment, and euthanasia of animals.

Conflict of interests

The authors declare that they have no conflict of interests.

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