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Effect of Different Doses of Curcumin on Sperm Parameters and Oxidative Stress in Testis of D-Galactose Induced Aging Mice Model

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ABSTRACT

BACKGROUND AND OBJECTIVE: Aging is accompanied with low concentration of testosterone hormone and apoptosis induction in the testis. The aim of this research was to investigate the effects of different doses of curcumin as the active ingredient of Curcuma Longa Turmeric, on sperm quality and oxidative stress in mice D-galactose-induced aging model.

METHODS: 48 Balb/c mice (n=8) were randomly assigned to 6 groups: control, Sham, D-galactose and Curcumin 1 to 3 groups. 300 mg/kg of D-galactose was injected to D-galactose group. Curcumin 1 to 3 were injected D-galactose+25, 50 and 100 mg/kg curcumin intraperitoneally. Then, the oxidative stress based on biochemical parameters and sperm analysis according to WHO guideline were evaluated on day 42 of the experiment.

FINDINGS: Mean sperm count in control group was (4.17±0.84) while it reduced after reception of 300 mg/kg D-galactose (3.06±0.86). There was a significant increase in the sperm parameters in Curcumin group compared to the D-galactose group ($p<0.05$). A significant increase was observed in the level of thiol and superoxide dismutase enzyme in curcumin group 3, compared to the D-galactose group ($p\leq0.001$). Significant decreases in catalase and malondialdehyde enzymes were observed in the D-galactose group, compared to the curcumin 1, curcumin 2 and curcumin 3 groups ($p<0.05$).

CONCLUSION: Administration of curcumin for 2 weeks improved sperm parameters and decreased oxidative stress in testis of mice D-galactose-induced aging model.

KEY WORDS: *Curcumin, Aging, Sperm, Mice*.

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Introduction

The aging process is a biological phenomenon that gradually reduces the effectiveness of many physiological actions of the body and the body's homeostasis, and the damaging materials accumulate in the cell. As the age increases, decreasing the testosterone secretion, decreasing the antioxidant defense system of the body with increasing apoptosis and increasing the level of free radicals (1, 2). The aging phenomenon affects various body organs, including the nervous system, urinary system and genitalia. Various factors such as environmental conditions and nutrition have a significant impact on the process. There are over 300 theories for the aging process, many of which have overlap. One of the most prominent of these is the theory of free radicals that it is suggested in the theory that during an aging phenomenon, the antioxidant defense system of the body is not able to neutralize all produced free radicals, therefore, oxidative stress is created in the tissues, which leads to various diseases (1, 2). On the other hand, studies have shown that the aging process is associated with impairment of immune function, lowering the secretion of sex hormones and inducing apoptosis.

Curcumin is one of the natural polyphenols and the active ingredient of Curcuma longa, a yellow-orange color of turmeric is because of its existence. Studies have shown that curcumin has potent antioxidant potency and has no toxic effects on humans (3). In addition, curcumin has anti-inflammatory, antimicrobial, anti-cancer and anti-diabetic properties (4, 5). This substance has been shown to improve serum testosterone level, number and motility of sperm in metronidazole-treated mice (6). Cheraghi et al. reported protective effects of curcumin on acute toxicity of aluminum in the male reproductive system of rats (7). Curcumin protects the testicles and improves the quality of the Semen against the oxidative effects of di-phthalate (8). In another study, 20 micromoles of curcumin improved sperm motility and increased FSH levels after exposure to fluorescent lamps for 45 days (9). Administration 5 micromoles of curcumin in the in vitro environment increased motility, survival, and integrity of the sperm membrane (10).

Liao et al. also observed an increase of 40% in C. Elegans lifetime and reduction of lipophosin pigments due to administration of Mm20 curcumin (11). Considering the importance of the aging process and its impact on various biological systems in the body, it is essential to study the factors that can have protective effects in this process, because the study of the protective effects of curcumin on the aging process in the male reproductive system has not been studied. In this study, an aging model developed with D-galactose, one of the most accepted methods for aging in animal models, was used (12). The purpose of this study was to investigate the effects of different dose of curcumin on sperm parameters and oxidative stress in mice of D-galactose induced aging mice model.

Methods

In this experimental study, after approval by the Ethics Committee of the Mashhad University of Medical Sciences, with code of 466.1396IR.MUMS.fm.REC., 48 adult male Balb/C mice were provided from Animal Hospital of the Medical University and were kept under standard conditions (room temperature22 ±2 ° C, humidity 50±5%, optical cycle 12 hours of darkness and 12 hours of brightness). Animals enjoy free access to water and food throughout the study. The mice were randomly divided into six groups (control, sham, D-galactose, curcumin 1 to 3).

The control group received no injections. The sham group received D-galactose (normal saline+DMSO) as an intraperitoneal agent. D-galactose group received 300 mg/kg D-galactose for 6 weeks and received intraperitoneal injection. The group of curcumin 1 (D-galactose+25 mg/kg curcumin), curcumin group 2 (D-galactosum+50 mg/kg curcumin) and curcumin group 3 (D-galactosum+100 mg/kg curcumin) were intraperitoneally administered for 14 days (6 and 13). Then, sperm analysis and oxidative stress levels were evaluated on day 42 of the experiment. Given that 42 days were needed to create an aging model, day 42 was selected for sampling.

Sperm quality assessment: In order to evaluate the sperm parameters, after 42 days, the chopped pieces of epididymis were placed in normal saline and placed in a CO2 incubator for 30 to 45 minutes. Then, mobility, sperm count and morphology of sperm were evaluated based on WHO guidelines. In addition, to investigate the survival of sperm, eosin staining was used (14, 15)

Measurement of thiol group's levels: Tris buffer was adedto 50 µL of a homogenized solution of testicular tissue and absorption was read at 412 nm using a spectrophotometer (A1). Then, 20 µl of the reagent was added to the dithio nitrobenzoic acid, and after 15 minutes incubated at room temperature, the absorbance was again investigated at 412 nm (A2). Blank absorption was considered as B, the following formula

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was used to calculate the thiol concentration in micromoles per gram (16).

(A2-A1-B) × 1.07/0.05 × 13.6

Malondialdehyde Level Measurement: 1 ml of homogenized testicular tissue was mixed with tri-chloroacetic acid chloride and put 45 minutes in a boiling water bath. After cooling, it was centrifuged at 1000 rpm for 10 minutes and absorption was read at 532 nm using a spectrophotometer and the concentration of malondialdehyde was calculated in nmol/g (16).

Catalase enzyme level Measurement: In summary, 30 M hydrogen peroxide was added to the homogenous tissue sample in 50 mM sodium phosphate buffer. The specimen absorption was read using spectrophotometer at 240 nm (17).

Measurement of superoxide dismutase level: measurement of this enzyme was carried out using the method of madesh et al. After homogenizing the tissue in 10 mM Phosphate buffer at pH=4/4, standard enzyme solutions were prepared with different concentrations and a standard curve was prepared. Then, 65 µl of phosphate saline buffer, 30 µl (MTT 25.1 mM) and 75 µL of pyrogallol (100 µM) with 10 µl of homogenized tissue were mixed and incubated for 10 minutes at room temperature. Subsequently, 75 µl of dimethylsulfide oxide was added and absorption was read at 570 nm using an ELISA device (18).

Statistical analysis: Data were analyzed using SPSS software and one-way ANOVA and Tukey's post hoc test. P<0.05 was considered significant.

Results

Results of sperm parameters: The average number of sperm in millions per ml in the control group (4.17±0.84), while after receiving 300 mg/kg of D-galactose, the number of sperm significantly decreased (3.06± 0.86). The number of sperm in all groups receiving curcumin (p≤0.001) was significantly higher than that of D-galactose group (Table 1). Statistical analysis did not show a significant difference between the number of sperm in the different groups of curcumin recipients (p<0.05). Mean percentage of sperm motility was 82.8±9.72 in the control group, while it decreased significantly (51.25±12.51) after the administration of D-galactose. Statistical analysis showed a significant difference between sperm motility in curcumin group 1 (p≤0.001), curcumin group 2 (p≤0.001), curcumin group 3 (p≤0.001), control group (p≤0.001) and sham group (p≤0.001) that were higher than D-galactose group. The lowest rates of mobility were observed in the D-galactose group (51.25%) and the highest mobility was found among the groups receiving curcumin in the curcumin group 3 (81.5%). Infusion of D-galactose resulted in a significant decrease in the natural morphology of sperm compared to the control group (p=0.001), which ranged from 82.75±7.62 to 61.75±12.33. Administration of curcumin in all three doses corrected this decrease and resulted in a significant increase in the natural morphology of sperm compared to the D-galactose group (p<0.05). Among the groups that received the curcumin, the highest natural morphology of sperm (80.5%) was obtained in the group receiving 50 mg/kg. Statistical analysis showed a significant difference between the percentage of sperm survival in the curcumin group1 (p=0.008), curcumin group 2 (p=0.006), curcumin group 3 (p=0.001), control group (p≤0.001) and Sham group (p=0.001) compared to D-galactose group. With the administration of 100 mg/kg of curcumin, the highest percentage of survival (82.25%) was observed.

Thiol levels results: Showed a significant difference between the mean thiol level in D-galactose group (p≤0.001), Curcumin group 1 (p≤0.001), Curcumin group 2 (p≤0.001) and Curcumin group 3 (0.003) 0p =). in comparison with the control and Sham groups. Significant increase was observed in the level of thiol of curcumin group 3 (p=0.000) compared to D-galactosin groups (p≤0.001), curcumin1 (p≤0.001) and curcumin2 (p≤0.001) (Fig 1).

Maladaldehyde levels (MDA): The mean malondialdehyde level in the control group was 0.52±0.1 nmol/g, while in the D-galactose group it increased to 29.2±1.55. There was a significant difference between the mean level of malondialdehyde in D-galactose group (p≤0.001), Curcumin group 1 (p=0.001) and Curcumin group 2 (p=0.002) compared to Control and Sham group. In addition, there was a significant difference in the groups of curcumin group1(p=0.01), curcumin group2 (p=0.006) and curcumin group3 (p≤ 0.001) compared to D-galactose group (Fig 2).

Levels of SOD and CAT: The mean level of superoxide dismutase enzyme level in the control group was 2.38±0.26 kg/g, while in the D-galactose group it decreased to 0.28±0.23. The statistical test showed a significant increase in the mean level of superoxide dismutase enzyme between sham group and control with D-galactose group (p≤0.001), curcumin group 1 (p≤0.001) and curcumin group 2 (p≤0.001). Also, there

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was a significant increase between the mean superoxide dismutase level in the curcumin group 3 with curcumin group 1 ($p\leq0.001$), curcumin group 2 ($p=0.002$) and D-galactose group ($p\leq0.001$) (Fig 3). The mean level of catalase enzyme in the control group was 0.06±0.12 units/g while in the D-galactose group it decreased to 0.01±0.001. The statistical test showed a significant difference between the mean level of catalase enzyme in control group and sham with D-galactose group ($p\leq0.001$), curcumin group 1 ($p\leq0.001$) and curcumin group 3 ($p\leq0.001$). Also, there was a significant decrease in the mean level of catalase enzyme in D-galactose group compared to curcumin group 1 ($p\leq0.001$), curcumin group 2 ($p\leq0.001$) and curcumin group 3 ($p\leq0.001$). Significant increase was observed between the mean level of catalase enzyme in curcumin group 3 compared to curcumin group1 ($p\leq0.001$) and curcumin group2 ($p\leq0.001$). In addition, there was a significant increase between the mean level of catalase enzyme in curcumin group2 compared to curcumin group1 ($p\leq0.001$).

Table1. Effect of administration of curcumin on sperm parameters (number, mobility, morphology and survival) in the different groups

Group	Sperm count (Million/ml)	Sperm mobility(%) Mean±SD	Natural sperm morphology (%) Mean±SD	Sperm survival(%) Mean±SD
Control	4.17±0.84#	82.5±9.72#	82.75±7.62#	85.75±7.22#
Sham	4.1±0.63#	82.5±8.79#	83.37±7.4#	82.12±8.64#
D-galactose	3.06±0.86*	51.25±12.51*	61.75±12.53*	61.75±15.39*
curcumin 1	4.74±0.54#	77.87±11.48 #	78.25±6.6#	79±8.64#
curcumin 2	4.61±0.49#	78±11.92#	80.5±10.12#	79.62±6.47#
curcumin 3	4.58±0.58#	81.5±10.86#	77.5±10.3#	82.25±7.4#

($p<0.05$) *: There was a significant difference with the control group in the same column, ($p<0.05$) #: a significant difference with the D-galactose group in the same column

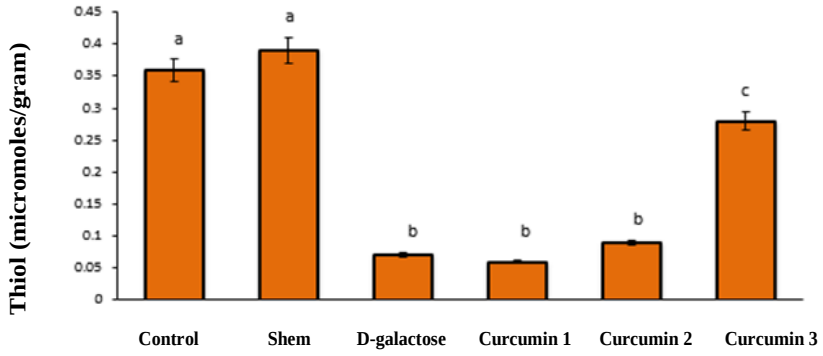

Figure 1. Comparison of thiol level in testicular tissue of different experimental groups

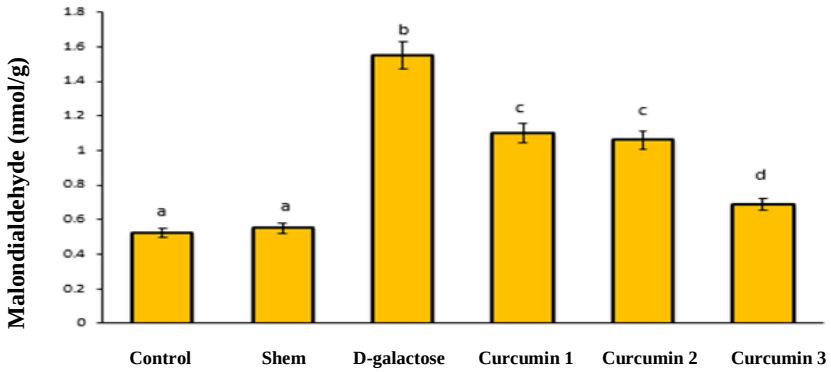

Figure 2. Comparison of malondialdehyde level (MDA) in testicular tissue of different experimental groups

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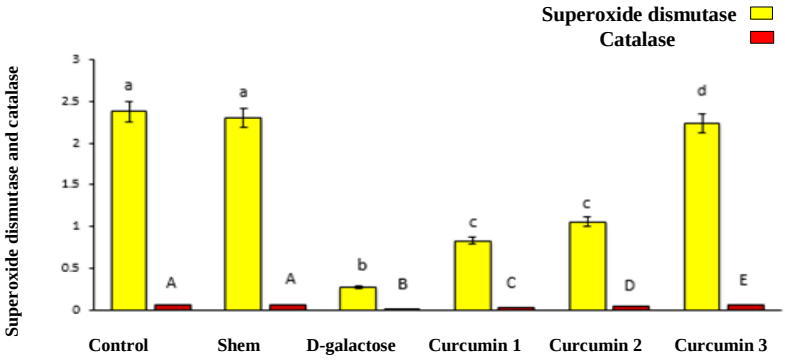

Figure 3. Comparison of superoxide dismutase and catalase levels in testicle tissue in different experimental groups

Discussion

In this study, curcumin in all three doses compensated the reduced spermatic parameter resulting from the administration of D-galactose and resulted in an increase in the sperm parameters compared to the D-galactose group. In the process of spermatogenesis of the mouse, the complete maturity of the spermatid occurs in 16 stages, in which spermatid is transformed into adult spermatozoa, because mature sperm appears after 13.5 days in the lumen of the seminiferous tubules. In this study, the rats received intraperitoneal curcumin for 14 days (19). In this study, the effects of curcumin on the reproductive system were dose dependent. Therefore, antioxidants are a double-edged sword which dosage and timing have the desired effect, if the choice of dose and inappropriate duration lead to reversible effects (20).

Tvrdá et al. observed that different doses of curcumin in the in vitro environment prevented oxidative stress and inhibited free radicals in male cow (21). Oral administration of 100 mg/kg curcumin for 28 days reduced the toxic effects of imidacloprid insecticide and improved sperm parameters, oxidative stress markers and histopathological changes in the testicle in the curcumin treated group (22). In the present study, there was a significant difference in the level of thiol and superoxide dismutase enzyme in the group receiving 100 mg/kg of curcumin dose compared to the D-galactose group . In addition, the level of catalase and malondialdehyde enzyme in the D-galactose group was significantly lower than that of the curcumin recipient group. One study showed that pre-treatment with curcumin reduced the percentage of abnormal morphology of sperm and serum levels of thiobarbituric acid in mice receiving metronidazole and 0.5 grey x-ray and increased the level of glutathione peroxidase and superoxide dismutase enzymes (23).The results of Chandra et al. Showed that in the group treated with curcumin, the histopathology of the testes, sperm count, testosterone levels, testicular index and superoxide dismutase levels were improved compared to the Chromium group (24). Concomitant with these studies, our study also found that the appropriate dose of curcumin improves sperm parameters and increases the antioxidant levels of superoxide dismutase and catalase. In addition, the level of malondialdehyde is significantly reduced. Administration of 200 mg/kg of curcumin also had protective effects on the elliptic toxicity of dexamethasone and increased the expression of Bcl2 gene, improved spermatogenesis, and testicular histopathology (25). Improvement of sperm parameters, testicular histopathology, reduction of malondialdehyde level and positive Tunel cells following intraperitoneal injection of 15 mg/kg of curcumin for 35 days in rats exposed to sodium arsenate were reported. However, there was no change in testicular index in the tested groups (26). Administration of 100 mg/kg of curcumin for 4 weeks improved tubule diameter, serum testosterone levels and decreased apoptotic cells compared with cadmium group (13). Treatment with 100 mg/kg curcumin for 8 weeks improved testicular damage in diabetic rats and decreased apoptotic cells compared with diabetic group (27).

Mu and colleagues reported positive effects of curcumin on spermatogenesis disorders caused by high fat diets in rats. In the curcumin recipient group, reduced the expression of apoptotic genes and also the number of testicular positive Tunel cells (28). Concerning the possible mechanism of curcumin effects, curcumin seems to affect apoptosis with effects on genes involved in apoptosis, such as Bcl2 and Bax genes (4, 29). In addition, curcumin affects the level of malondialdehyde and the body's antioxidant system, which reduces oxidative stress. In this study, it was better to use mice that were naturally aged instead of the mouse model of aging, which was not possible due to the lack of availability of storage conditions. In addition, the expression of effective genes in apoptosis should also

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be investigated, which researchers are recommended for future studies. In addition, the effects of nano-curcumin and other modified curcumin derivatives are recommended. Administration of curcumin for 14 days improves sperm parameters and biochemical markers in the testicular tissue of an aging animal model.

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