

Protective Effect of Curcumin Against Nicotine-induced Damage on Reproductive Parameters in Male Mice

Efecto Protector de la Curcumina contra el Daño Inducido por la Nicotina sobre los Parámetros Reproductivos en Ratones Machos

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SUMMARY: Nicotine consumption can decrease fertility drive in males through inducing oxidative stress and DNA damage. The color of turmeric is because of a substance called curcumin for which some anti-oxidative and anti-inflammatory properties have been identified. In this study, various doses of curcumin (10, 30 and 60 mg/kg) and curcumin plus nicotine (10, 30 and 60 mg/kg) were administered intraperitoneally to male mice for 28 consequent days and reproductive parameters were determined. The results indicated that nicotine administration (0.5 mg/kg) significantly decreased testosterone level, count and motility of sperms, and testis weight compared to control group. However, increasing the dose of curcumin significantly increased reproductive indices in most of the groups. Thus, it seems that curcumin inhibits nicotine-induced adverse effects on reproductive parameters.

KEY WORDS: Curcumin; Nicotine; Reproductive Parameters; Mice.

INTRODUCTION

Infertility is a health problem that causes adverse effects in personal, social and economic domains and is observed in 10 to 15% of the couples (Ikechebelu *et al.*, 2003). About 40% of infertility problems are associated with men (Razzak & Wais, 2002). Infertility in males has been associated with sperm dysfunctions such as low sperm count, immaturity, abnormality and lack of motility (Araoye, 2003). Various studies have shown that consumption of nicotine-containing compounds decrease the sperm count and motility (Saleh *et al.*, 2002). Nicotine is a highly toxic organic compound containing nitrogen and alkaloid which is mostly found in tobacco (Jana *et al.*, 2010). Nicotine can easily pass through the cell membrane and react to tubulin protein present in the cytoplasm of multiplying cells and cause cell division disorder (Gorrod, 1993). Previous studies have indicated that nicotine can damage sperm membrane and DNA and induce apoptosis in interstitial cells in testis (Racowsky & Kaufman, 2008). Turmeric (Curcuma longa), one of the oldest plants, belongs to the family of *Zingiberaceae* family which has long been used in traditional medicine for blood purification, digestion, arthritis treatment, liver protection and as an anti-inflammatory agent. The co-

lor of turmeric is because of a chemical called curcumin (C₂₁H₂₀O₆) which comprises 3 to 4% of it (Agarwal *et al.*, 2008). Numerous studies have reported the anti-oxidant properties, anti-mutation and anti-tumor effects, and carcinogenic characteristic for curcumin (Daniel *et al.*, 2004). Curcumin affects the metabolism of arachidonic acid by inhibiting the phosphorylation of phospholipase A2 (PLA2), decreasing the expression of COX-2 gene and inhibiting the catabolic activity of COX-5. These effects induce the anti-inflammatory activity of curcumin. In addition, curcumin decreases the expression of different inflammatory cytokines such as IL-1, TNF α, IL6 and chemokines (Chigurupati *et al.*, 2008). El-Wakf *et al.* (2011) indicated that curcumin can be useful in the treatment of male infertility owing to oligospermia and decreasing male sexual hormones. Although researchers have reported the anti-oxidant properties of curcumin, its effects on reproductive hormones, epididymis pathway, and sperm count and motility in nicotine-infected rats have not been investigated. Therefore, the present study was conducted to analyze the protective effect of curcumin on the damage induced by nicotine in reproductive parameters in male mice.

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MATERIAL AND METHOD

Curcumin and Nicotine preparation. Curcumin powder ($C_{21}H_{20}O_6$) was purchased from Merk company (Merk-Germany). The powder was dissolved in ethanol 70% (C_2H_5OH) and diluted by normal saline to prepare different doses. Also, the nicotine solution ($C_{10}H_{14}N_2$) was purchased from Merk Company (Merk-Germany). This solution was diluted by normal saline for administration.

Animals. Fifty six male mice with the weight range of 25–30 g were purchased from Tehran Razi Institute. The animals were kept in the temperature of $22\pm 2^\circ C$, under controlled environmental conditions, 12/12 h light/dark cycle and free access to water and food (Gebreegziabher *et al.*, 2004).

Animal classification. The mice were randomly assigned to 8 groups ($n=7$). The control group was administered ethanol (1 mL/kg) and experimental groups were administered nicotine (0.5 mL/kg), curcumin (10, 30 and 60 mg/kg) and curcumin (10, 30 and 60 mg/kg) plus nicotine (0.5 mL/kg) for 28 consequent days. The nicotine (0.5 mL/kg) group, while being compared to ethanol (1 mL/kg) (control) group, was used as a secondary control group for the groups that simultaneously received curcumin and nicotine (Bancroft & Gamble, 2002).

Analysis of testis weight and testosterone hormone. The animals were anesthetized 24 h after the last injection. Blood was taken from the heart and preserved in the temperature of $37^\circ C$ for 30 minutes and was centrifuged (1000 g) for 15 minutes. Its serum was collected and preserved in $-20^\circ C$ until the measurement of testosterone hormone. Testosterone hormone was measured by ELISA method. The testes were separated and weighed separately and their means were used. Then, testes were preserved in neutral buffered formalin 10% (Lotfi *et al.*, 2013).

Evaluation of sperm characteristics. Cauda epididymis was separated into small segments and placed in the medium DMEM/F12 containing FBS 5% which had been previously balanced in the incubator with the temperature of $37^\circ C$ and CO_2 5%. The prepared suspension was used for the analysis of sperm parameters including: motility, count and morphology. Sperm motility was divided into four levels: (0): without motility, (I): minor in situ motility. (II): circumferential motility and (III): progressive motility (Mehrabi nasab *et al.*, 2010).

Morphologic analysis of sperm count and motility. To count the sperms, after putting the sperm suspension on Neubauer's chamber, the sperms on the four corners of the central square

were counted. To examine the sperm morphology, smear was prepared from the samples and was stained and investigated by Papanicolaou method. To determine the motility, one drop of the sperm suspension was placed on the chamber and the motile and immotile sperms were analyzed by microscope with magnification 40x (Anass & Ahmed, 2013).

Histological analysis. Having fixed the testes, the tissue processing, including dehydration, clearing and embedding was performed. Microscopic sections ($5\ \mu m$) were prepared and stained by H&E method. More than 20 sections were prepared from each block. The sections numbered 5, 10, 15, and 20 were selected and photographed separately from three random scopes. Seminiferous tubules diameter was measured by Motic camera and software (Moticam 2000, Spain). The mean of seminiferous tubules diameter (μm) was determined for each testis (Lotfi *et al.*) (Fig. 1).

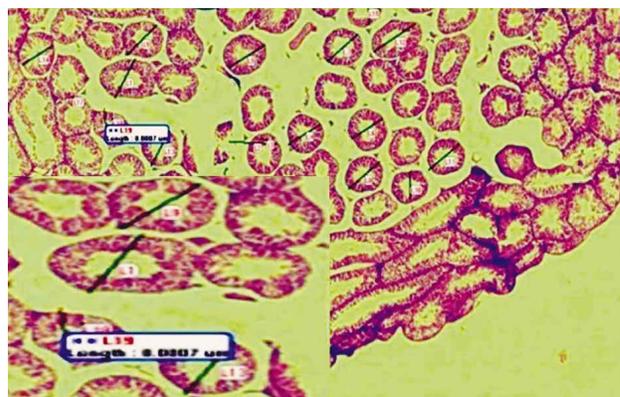


Fig. 1. Analysis of seminiferous tubules diameter by Motic camera and software. Effect of curcumin (10 mL/kg). Magnification (10x).

Statistical analysis. All the quantitative data were presented as Mean \pm Standard Deviation. One-way analysis of variance (ANOVA) followed by LSD post-hoc test were performed to determine the statistical significance between different groups using SPSS software package 16.0. $P < 0.05$ was considered significant.

RESULTS

The effective dose of nicotine (0.5 mL/kg) caused a significant decrease in the testis weight of the mice compared to control (ethanol-saline) group ($p < 0.05$). By increasing the dose, curcumin increased the testis weight, which was reported significant in 30 and 60 mg/kg doses in comparison with control (ethanol-saline) group ($p < 0.05$). Further, curcumin-nicotine, by increasing the dose, significantly

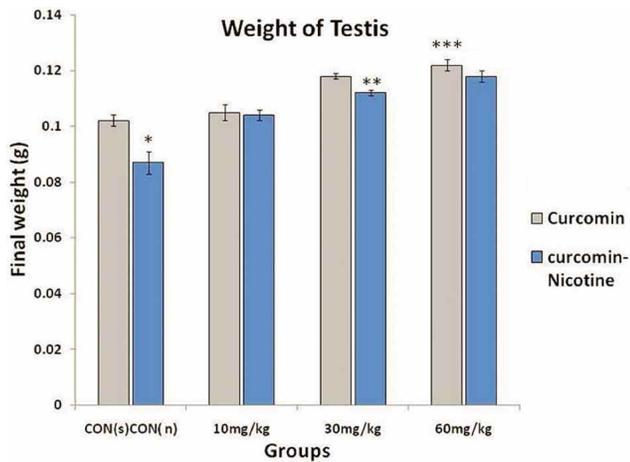


Fig. 2. Analysis of the effect of nicotine, curcumin and curcumin-nicotine on testis weight.

CON(n)= Effect of nicotine (0.5 mL/kg) on testis weight considered as control for curcumin-nicotine group as well. CON(s)= Control group with saline administration. *= Significant decrease of testis weight in nicotine group compared to saline group ($p < 0.05$). **= Significant increase in all groups compared to control group ($P < 0.05$). ***= Significant increase in 30 and 60 mg/kg groups compared to control group ($P < 0.05$).

increased the testis weight in all groups compared to control (nicotine) group ($p < 0.05$) (Fig. 2).

Further, nicotine (0.5 mL/kg) significantly decreased the motility, count and normal morphology of sperms in comparison with control (ethanol-saline) group ($p < 0.05$). Moreover, by increasing the dose, curcumin and curcumin-nicotine significantly increased the motility, count and normal morphology of sperms in all groups compared to control (nicotine) group ($p < 0.05$) (Figs. 3 and 4).

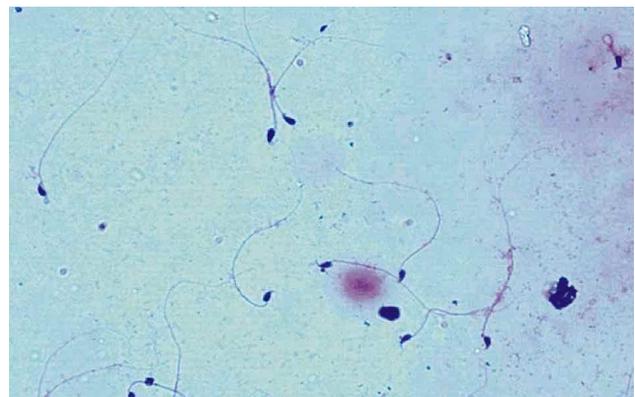


Fig. 4. Morphological analysis of sperms affected by nicotine (10 mL/kg). Separated head, crooked head, crooked tail, crooked neck and no hook head as a result of nicotine administration were observed in sperms (magnification 40x).

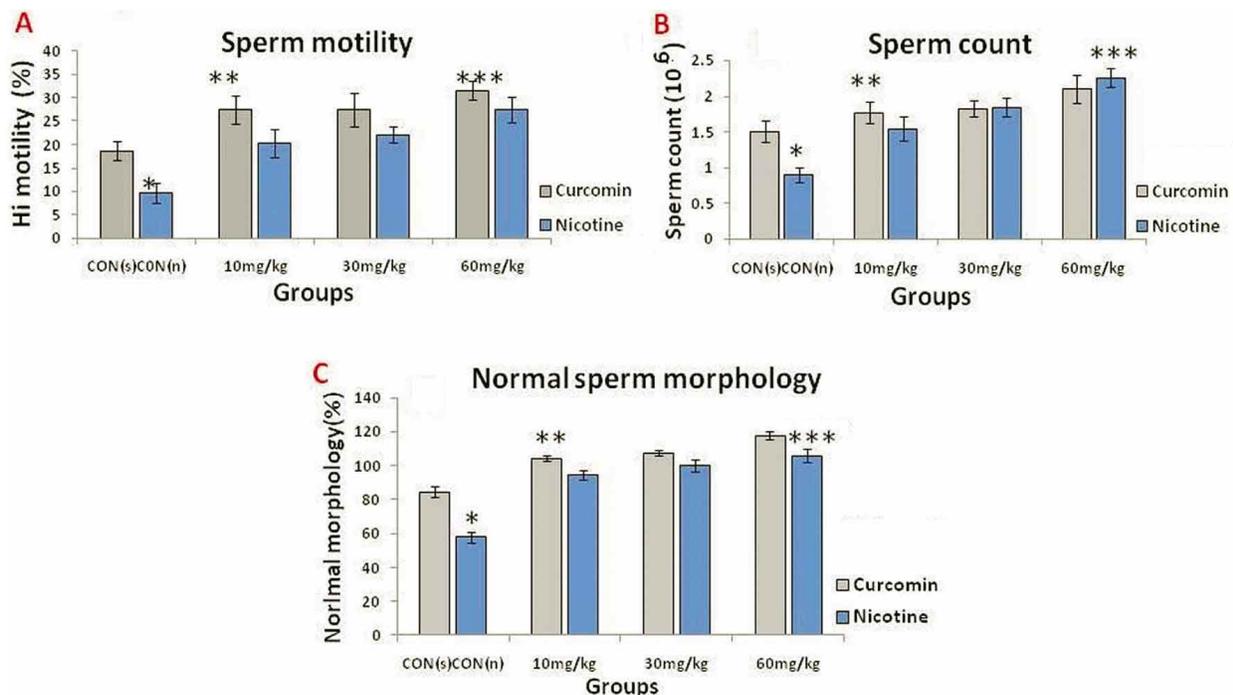


Fig. 3. Analysis of the effect of nicotine, curcumin and curcumin-nicotine on sperm motility (A), sperm count (B) and sperm normal morphology (C). CON(n)= Effect of nicotine (0.5 mL/kg) on testis weight considered as control for curcumin-nicotine group as well. CON(s)= Control group with saline administration. *= Significant decrease of testis weight in nicotine group compared to saline group ($p < 0.05$). **= Significant increase in all groups compared to control group ($P < 0.05$). ***= Significant increase in all groups compared to control group ($P < 0.05$).

In addition, nicotine (0.5 mL/kg) caused a significant decrease in the seminiferous tubules diameter in comparison with control (ethanol-saline) group ($p < 0.05$). By increasing the dose, however, curcumin and curcumin-nicotine significantly increased seminiferous tubules diameter in all groups ($p < 0.05$) (Fig. 5). Also, nicotine (0.5 ml/kg) significantly decreased testosterone hormone compared to control (ethanol-saline) group ($p < 0.05$). By increasing the dose, curcumin and curcumin-nicotine significantly increased testosterone hormone in all groups ($p < 0.05$) (Fig. 6).

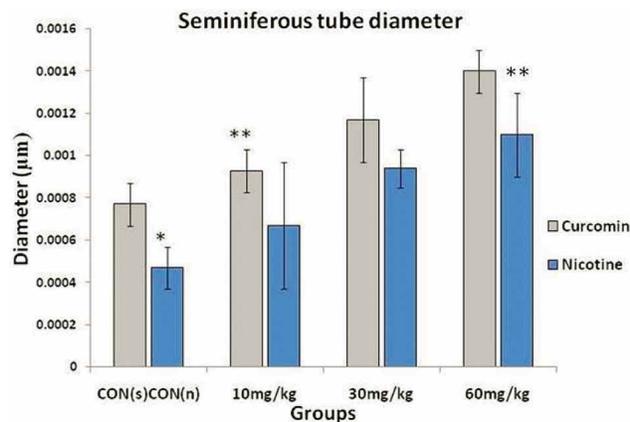


Fig. 5. Analysis of the effect of nicotine, curcumin and curcumin-nicotine on seminiferous tubules diameter. CON(n)= Effect of nicotine (0.5 mL/kg) considered as control for curcumin-nicotine groups as well. CON(s)= Control group with saline administration. *= Significant decrease in nicotine group compared to saline group ($P < 0.05$). **= Significant increase in all groups by increasing the dose ($P < 0.05$).

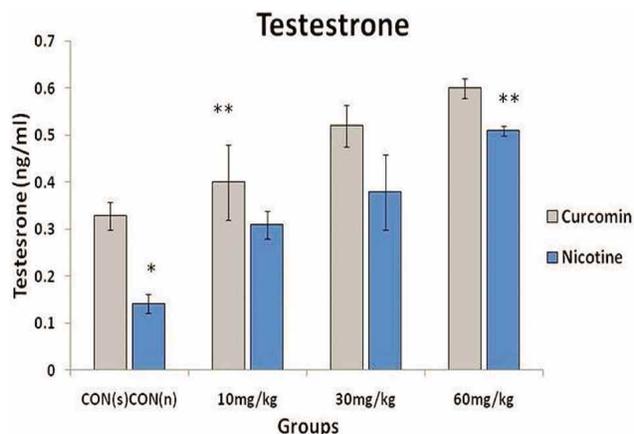


Fig. 6. Analysis of the effect of nicotine, curcumin and curcumin-nicotine on testosterone hormone. CON(n)= Effect of nicotine (0.5 mL/kg) considered as control for curcumin-nicotine groups as well. CON(s)= Control group with saline administration. *= Significant decrease in nicotine group compared to saline group ($P < 0.05$). **= Significant increase in all groups by increasing the dose ($P < 0.05$).

DISCUSSION

The most important finding of the present study was the harmful effect resulting from exposure to nicotine, especially on normal sperm count and motility in mice. In the present study, the intraperitoneal injection of nicotine resulted in the decrease of testosterone hormone and testis weight and impairment of reproductive variables. On the other hand, however, curcumin caused a significant change in these indices and inhibited the harmful effects induced by nicotine in the reproductive hormone. Nowadays, medicinal plants have numerous applications and one of the target tissues for plant extracts is reproductive organs such as testis and sperm parameters. It seems that curcumin increases the count and motility of normal sperms in treated groups through enhancing the anti-oxidant defense of the body (Valiko *et al.*, 2005). Curcumin can act as an anti-oxidant and improve the sperm quality by increasing the expression of anti-oxidant genes in comparison with nicotine group.

The findings obtained in this study are in line with the results of the study conducted by Kalpana *et al.* (2007), in which they investigated the relative peroxidative and anti-oxidant effects of curcumin on the nicotine-induced toxic fatty tissue. They reported that curcumin can decrease the toxicity induced by nicotine in the fatty tissue. Since the spermatogenic cells, owing to having high levels of unsaturated fatty acids, several dual links in plasma membrane and low levels of cytoplasmic antioxidants, are sensitive to oxidative damage (Rao *et al.*, 1989), oxidation of the membrane fatty acids will result in the loss of membrane fluidity and will decrease the activity of enzymes and ion channels of sperm. With regard to the fact that nicotine is one of the producers of reactive oxygen species, it seems that clearing oxidative agents by chemicals like curcumin can help cure and prevent the incidence of the diseases associated with sperm. The results of the present study confirm the findings of (Shang *et al.*, 2010), that indicated curcumin can be used as a potent anti-oxidant substance against oxidative stress and subsequent effects.

Sperm motility is an important factor in natural fertility and low sperm motility is the cause of most of the infertilities (Aitken, 1995). Increasing the sperm motility by curcumin in the present study may be due to the inhibition of cannabinoids' activity, which decreases sperm motility by activating CB1 receptors in mature sperm, by curcumin. The activity of these factors is probably increased by nicotine (Rossato *et al.*, 2005). The present study analyzed the effects of nicotine on testis weight decrease which might be associated with the appetite decreasing effects of nicotine that is overcome by curcumin appetizing property. Generally,

it seems that nicotine mainly induces oxidative stress and impairs reproductive tissue through producing free radicals. Further, anti-oxidant supplements such as curcumin can inhibit the harmful effects induced by nicotine on the hormones and quality and quantity of reproductive indices.

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JALILI, C.; KHANI, F.; SALAHSHOOR, M. R. & ROSHANKHAH, S. Efecto protector de la curcumina contra el daño inducido por la nicotina sobre los parámetros reproductivos en ratones machos. *Int. J. Morphol.*, 32(3):844-849, 2014.

RESUMEN: El consumo de nicotina puede disminuir la fertilidad en los hombres mediante la inducción de estrés oxidativo y daño del ADN. El color de la cúrcuma se debe a una sustancia llamada curcumina en la cual se han identificado algunas propiedades anti-oxidantes y anti-inflamatorias. En este estudio se administraron diferentes dosis de curcumina (10, 30 y 60 mg/kg) y de curcumina más nicotina (10, 30 y 60 mg/kg) por vía intraperitoneal a ratones machos durante 28 días consecutivos y se determinaron los parámetros reproductivos. La administración de nicotina (0,5 mg/kg) disminuyó significativamente el nivel de testosterona, el número y motilidad de los espermatozoides, y peso de los testículos en comparación con el grupo control. Sin embargo, el incremento de la dosis de curcumina aumentó significativamente los índices reproductivos en la mayoría de los grupos. Este estudio sugiere que la curcumina inhibe los efectos adversos inducidos por la nicotina sobre los parámetros reproductivos.

PALABRAS CLAVE: Curcumina; Nicotina; Parámetros Reproductivos; Ratón.

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