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Original Article

Ameliorative effect of curcumin on fluoxetine-induced reproductive toxicity and oxidative stress in male albino rats

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Bax; Curcumin; Fluoxetine; Lipid peroxidation; Testis

Abstract
Fluoxetine is a selective serotonin reuptake inhibitor used to treat depression and mood disorders. Curcumin, the major active ingredient of turmeric (*Curcuma longa*), showed many pharmacological effects and is known for its potent antioxidant capacity. The present work aims to evaluate the possible ameliorative effect of curcumin on fluoxetine-induced reproductive toxicity and oxidative stress in albino rats. Histological examination of testis of fluoxetine-treated rats revealed degeneration of spermatogenic cells, congestion of blood vessels and destruction of Leydig cells. In addition, speramatogenesis was inhibited as indicated by the decrease of the number of different spermatogenic cells. The diameters of the seminiferous tubules and heights of their germinal epithelium were significantly reduced. Moreover, significant higher numbers of Bax-positive Leydig cells were recorded. Testosterone, luteinizing hormone (LH) and glutathione (GSH) were decreased while malondialdehyde were increased in sera of fluoxetine-treated animals. Treating animals with fluoxetine followed by curcumin revealed an improvement in the histological changes observed in animals treated with fluoxetine. Moreover, curcumin treatment leads to decrease in the number of Bax-positive cells, increase in testosterone, LH, GSH, and decrease of lipid peroxidation. According to the present results, it can be concluded that combined treatment with fluoxetine and curcumin can improve the testicular abnormalities induced by fluoxetine. This effect of curcumin may be attributed to its antioxidant properties.

INTRODUCTION

Fluoxetine is a potent and selective serotonin reuptake inhibitor widely prescribed for treatment of neurological disorders such as depression and anxiety [1]. However its use was accompanied with some side effects such as lung damage [2], hepatotoxicity [3, 4] and cardiotoxicity [5].The effect of fluoxetine on male fertility and reproduction was studied by some authors. Long-term ingestion of fluoxetine caused a decrease in spermatogenesis and weights of reproductive organs (testes, epididymides, ventral prostrate and seminal vesicle) in rats. The hormonal assay also showed significant decrease in testosterone levels and FSH levels [6]. da Silva Jr. *et al* [7] reported that treating rats with fluoxetine during the suckling period caused a decrease in testes weights, Sertoli cell number and lowered the serum level of FSH.

Antioxidant enzymes, mainly superoxide dismutase (SOD) and catalase (CAT), are the first line of defense against free radical induced oxidative stress; SOD is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide, and CAT is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water. Antioxidants play a major role in preventing the formation of free radicals, which are responsible for many oxidative processes leading to ailments such as heart disease, inflammatory conditions, cancer and diabetes [8]. Many plants have been identified as having potential antioxidant activities. Curcumin, the yellow pigment isolated from the rhizome of turmeric **(***Curcuma longa*), is widely used as a spice and coloring agent in foods. It has been used in traditional medicine as a stomach tonic and blood purifier, and for the treatment of skin disease and

wound healing [9]. Many studies showed that curcumin possesses anti-inflammatory [10], antioxidant [11], anti-tumor [12], hepatoprotective [13] and immune-modulatory [14] activities. The present study aimed to evaluate the ameliorative effect of curcumin against fluoxetine induced testicular damage and oxidative stress in albino rats.

MATERIALS AND METHODS

Fluoxetine

Fluoxetine (Philozac®) was obtained as capsules from Amoun Pharmaceutical Company, Cairo, Egypt. Each capsule contained 10 mg fluoxetine hydrochloride. Fluoxetine was dissolved in distilled water and was given orally to animals by gavage at a dose level of 10 mg/kg body weight (equivalent to the therapeutic dose for human according to Paget & Barnes [15] daily for eight weeks.

Curcumin extract

Dry turmeric rhizomes of the plant *Curcuma longa* were purchased from a local market at Shebin El-kom, Menufia, Egypt. They were crashed into powder, dissolved in distilled water and orally given at a dose level of 150 mg/kg body weight daily for eight weeks [16].

Animals and treatments

Adult male albino rats (*Rattus norvegicus*), approximately three months old and weighing 130±5 g were used. The animals were kept in individual special rodents cages in the laboratory under constant condition of temperature (25±3ºC) with a natural dark/light cycle (12/12 h). Animals were maintained on a standard rodent diet, obtained from Egyptian company of Oils and Soap, Kafr-El-Zayat, Egypt, manufactured especially for laboratory purposes. The diet composed of 20% casein, 15% corn oil, 55% corn starch 5% salt mixture and 5% vitaminized starch. Water was available *ad libitum*. Maintenance of animals and experimental procedures was approved by the animal ethical committee in accordance with the guide for care and use of laboratory animals of Menoufia University, Egypt. Animals were divided into 4 groups:

-First group; served as controls.

-Second group; rats were orally administrated with curcumin at a dose level of 150 mg/kg body weight.

-Third group; animals were treated with fluoxetine at a dose level of 10 mg/kg body weight, daily for 8 weeks.

-Fourth group; animals were given fluoxetine together with curcumin (same doses) daily for 8 weeks.

Histological Study

After 8 weeks of experimental period, animals were sacrificed via decapitation, then they were dissected, testes were removed, weighed and fixed in Bouin's solution. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 µ thicknesses were cut using rotary microtome and mounted on clean slides. For histological examination, sections were stained with Ehrlich's hematoxylin and counterstained with eosin. Seminiferous tubules diameter and germinal epithelial height were measured from the spermatogenic cells on the inner surface of the basement membrane through the most advanced cell types lining the lumen of the tubules.

Immunohistochemical Study

From each testis block, 5 µ-thick sections were cut on neoprene-coated slides. The immunostaining was performed using the avidin-biotin complex (ABC) method and an automatic autostainer (Code-On Immuno/DNA Slide Stainer, Biotek, Santa Barbara, CA, USA). Slides were deparaffinized and blocked for endogenous peroxidase with 1.75% hydrogen peroxide in methanol for 20 min, antigen retrieval for 15 min using Citra Antigen Retrieval solution (BioGenex, Fremont, CA, USA) in 90ºC water bath for 30 min. The slides were allowed to cool for 20 min before continuing. Slides were then blocked by normal bovine serum for 5 min at 37ºC. The monoclonal antibody was applied overnight in humid medium at room temperature followed by the biotinylated secondary antibody for 15 min at 37ºC and the ABC complex for 15 min at 37ºC (VECTASTAIN® Elite ABC Kit; Vector Laboratories, Burlingame, CA, USA). Diamino-benzidine (DAB) was applied for 20 min at room temperature as chromogenic slides were counterstained with hematoxylin, dehydrated, and covered by coverslips. In negative control slides, the same system was applied with replacement of the antibody by diluted normal bovine serum. Bax immunostaining was performed using A3533 polyclonal rabbit-anti-human Ig fraction (DAKO, Glostrup, Denmark) at a dilution rate of 1:50.

Biochemical assays

For enzymes determination, blood samples were collected from the inferior vena cava and then centrifuged. Sera were obtained by centrifugation of the blood sample and stored at –20ºC. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product (malondialdhyde; MDA) according to Ohkawa *et al* [17]. Glutathione (GSH) was measured using the method of Beutler *et al* [18]. Testosterone and LH were determined using radioimmunoassay kits supplied by Diagnostic Products Corp. (Los Angeles, CA, USA) according to Maruyama *et al* [19].

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Statistical Analysis

Data were expressed as mean values and standard deviations, and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

Effect on body and testes weights

Treatment with fluoxetine significantly ($P < 0.05$) decreased the body and testis weights in comparison with control animals. Co-administration with curcumin abolished this decrease ($P < 0.05$). Insignificant increase in body and testis weights was recorded in animals given curcumin in comparison with controls (Table 1).

Biochemical results

In fluoxetine-treated rats MDA levels were significantly ($P < 0.05$) increased compared with the control group (Fig.1a); the mean values were 83.7 ± 2.5 and 191 ± 5.5 nmol/ml in control and fluoxetine groups, respectively. In curcumin alone and the curcumin plus fluoxetine groups, the levels of MDA did not significantly differ from those of the control group. On the other hand, fluoxetine-treated rats showed significant decrease in GSH level and the mean value was 0.05 ± 0.009 µmol/sec/ml. Animals co-administered with curcumin showed an elevation in GSH level (Fig.1b). With respect to the change in reproductive hormones, the levels of testosterone and LH dramatically decreased in fluoxetine-treated rats (Fig.2). There is no significant difference ($P > 0.05$) in these hormones levels between curcumin, curcumin plus fluoxetine and control groups.

Histopathology and expression of bax

Histological examination of testis of control rats revealed a picture of full spermatogenic activity. The germ cells and Sertoli cells within the seminiferous tubules were normal. No histological alterations were observed in animals treated with curcumin. Treating animals with fluoxetine induced degeneration of the germ cells within the seminiferous tubules which appeared with large vacuoles (Fig.3).

Table 1. Change in body and testes weights in different animal groups

Treatment group	Body weight	Testis weight
Control	174 ± 8.09	1.216 ± 0.12
Curcumin	176 ± 5.5	1.252 ± 0.34
Fluoxetine	130 ± 4.4*	1.19 ± 0.2*
Fluoxetine + Curcumin	170 ± 5.3	1.36 ± 0.29

*****Significant at $P < 0.05$

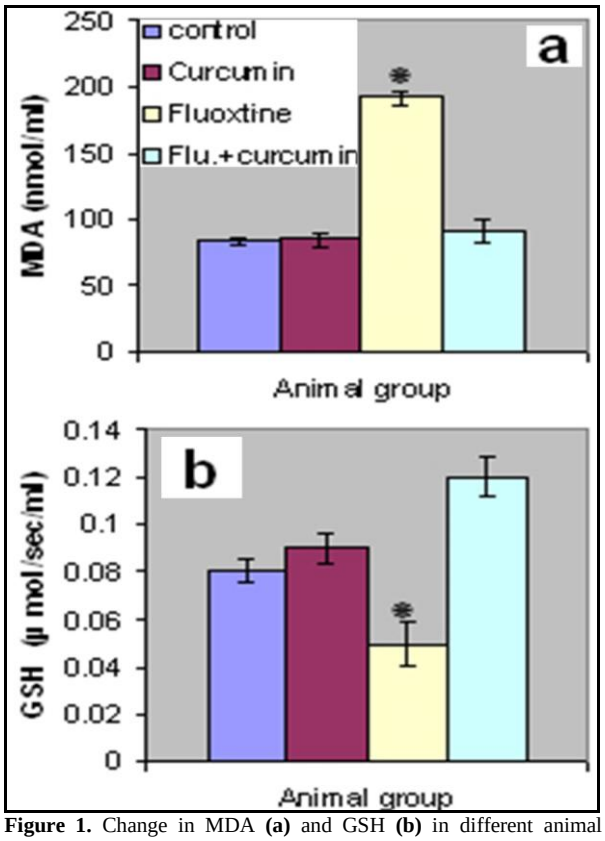

Figure 1. Change in MDA **(a)** and GSH **(b)** in different animal groups; *****significant at $P < 0.05$.

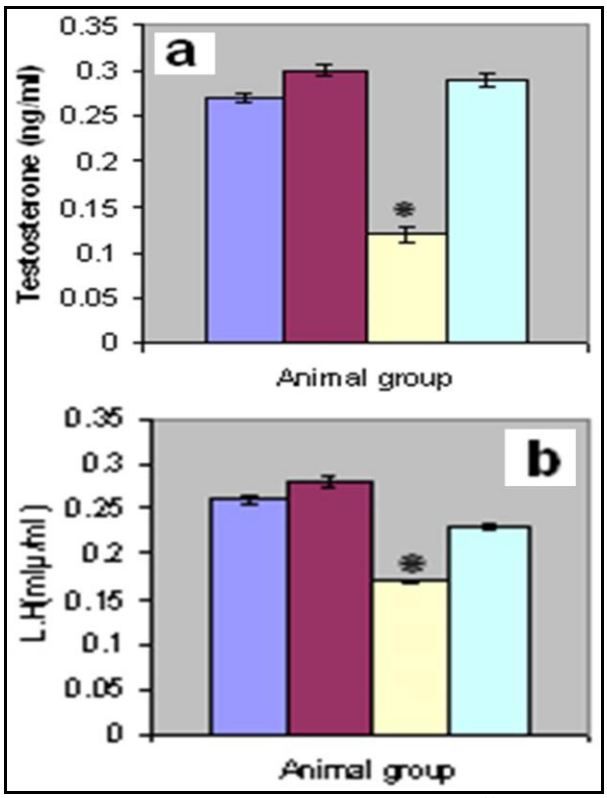

Figure 2. Change in testosterone **(a)** and LH **(b)** in different animal groups; *significant at $P < 0.05$.

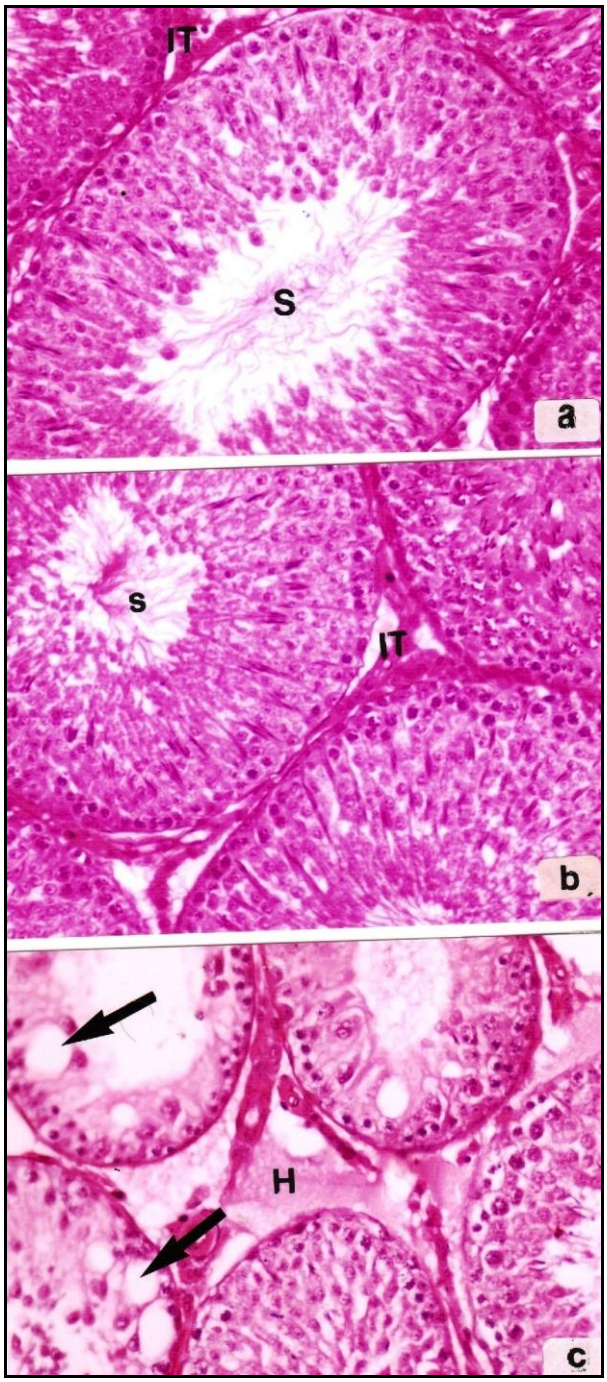

Figure 3. (a) Section in testis of a control rat. **(b)** Curcumin-treated rat showing normal seminiferous tubules; S: sperms, IT: interstitial tissue. **(c)** Section in testis of a rat treated with fluoxetine showing intertubular hemorrhage (H) and large vacuoles (arrow); x300.

Intertubular hemorrhage and degeneration of Leydig cells were observed. Many tubules showed few numbers of germ cells and the number of sperm decreased in some tubules (Fig.4a). This indicates inhibition of spermatogenesis. Animals treated with curcumin plus fluoxetine showed less degeneration of seminiferous tubules with an increase in the number of germ cells (Fig.4b).

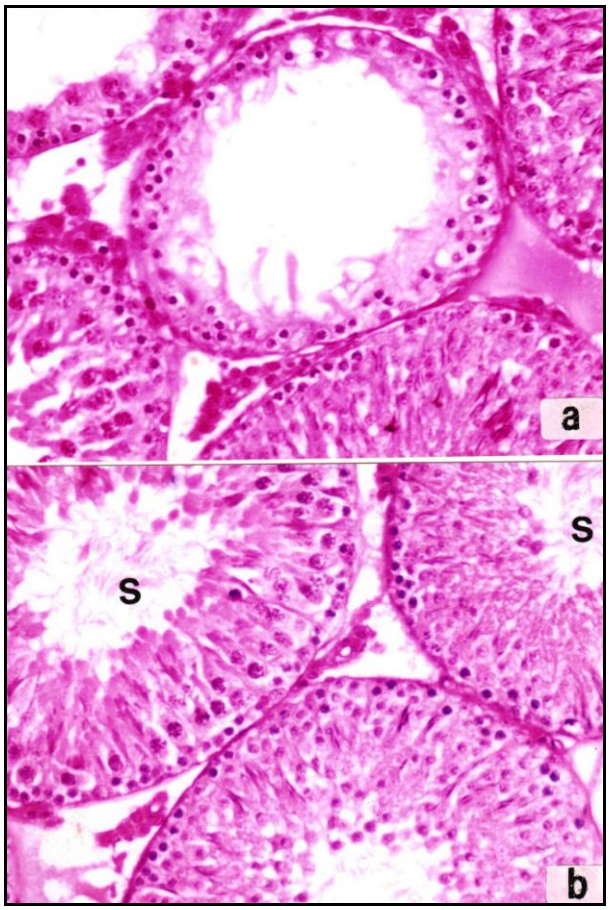

Figure 4. (a) Section in testis of a rat treated with fluoxetine showing inhibition of spermatogenesis. **(b)** Section in testis of a rat treated with fluoxetine and curcumin showing increase of spermatogenic cells, S: Sperms X 300.

Treating rats with fluoxetine for 8 weeks caused significant decrease ($P < 0.05$) in tubules diameters and their epithelial heights. On the other hand, animals treated with curcumin plus fluoxetine for the same period showed significant increase in the diameter of seminiferous tubules and their epithelial heights in comparison with fluoxetine group. No significant changes were recorded in tubules diameter or epithelial height of curcumin-treated rats compared with control animals (Fig.5). An increase in expression of Bax was observed in Leydig cells in rats given fluoxetine (Fig.6a). Treating animals with curcumin plus fluoxetine lead to significant decrease in Bax expression (Fig.6b). The percentage of bax positive cells is depicted in Fig.7.

DISCUSSION

The present results showed that fluoxetine decreased the body weight of the rats. This results correlates well with the results of Gutierrez *et al* [20] who reported that chronic fluoxetine administration in obese Zucker rats generated a reduction in body weight gain, food intake, adipocyte size, fat mass and body protein.

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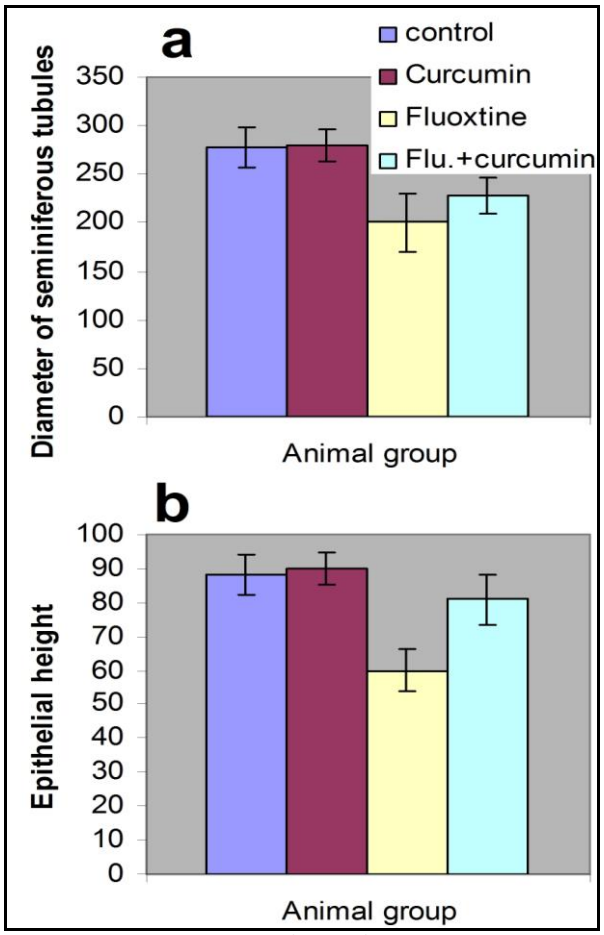

Figure 5. Change in diameter of seminiferous tubules **(a)** and their epithelial heights **(b)** in testes of different animal groups.

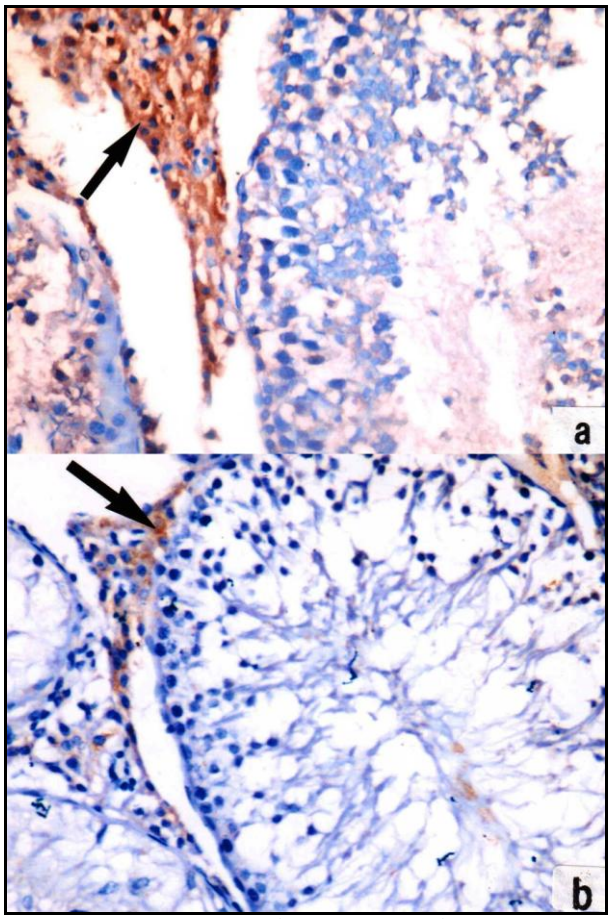

Figure 6. (a) Section in testis of a rat treated with fluoxetine showing increase of Bax expression in Leydig cells (arrow). **(b)** Section in testis of a rat treated with fluoxetine and curcumin showing decrease of Bax expression in Leydig cells (arrow); x300.

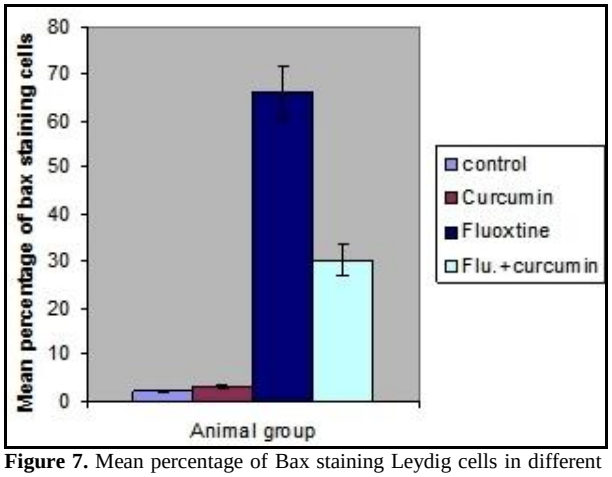

Figure 7. Mean percentage of Bax staining Leydig cells in different animal groups.

Rygula *et al* [21] examined the effects of fluoxetine on rats exposed to chronic stress. They found diminishing preference for sucrose solution paralleled by decreased body weight gain. Curzon *et al* [22] showed that fluoxetine reduces food intake and thus body weight in rats during sub-chronic and chronic treatment regimens, an effect apparently mediated by fluoxetine's impact on the serotonin (5-HT) signaling pathways.

Fluoxetine treatment was found to cause many histopathological changes in testes. Morphometric results indicated that fluoxetine caused significant decrease in seminiferous tubules diameters and their epithelial heights. Bataineh and Daradka [6] observed the same results in rats after long-term ingestion of fluoxetine for 60 days.

Aggarwal *et al* [23] reported that treating rats with fluoxetine caused distortion of seminiferous tubules, decreased thickness of germinal epithelium, decreased diameter of seminiferous tubules and decreased counts of germinal cell lineage. Gouvea *et al* [24] showed that maternal exposure to fluoxetine impaired sexual motivation in adult male mice.

Silva *et al* [7] concluded that neonatal administration of fluoxetine decreased final Sertoli cell number in Wistar rats. The authors observed that seminiferous epithelium and total length of seminiferous tubules showed a reduction in fluoxetine treated groups. A reduction in

the number of spermatogonia A and B were also observed. Immuonohistochemical examination of testes of rats treated with fluoxetine indicated that fluoxetine increased expression of Bax in Leydig cells. Djordjevic *et al* [25] reported that fluoxetine affected antioxidant system and promoted apoptotic signaling in Wistar rat liver. They added that fluoxetine induced several hallmarks of apoptosis in the liver, including a decrease in Bcl-2 expression and increased DNA fragmentation. However, apoptotic alterations were more pronounced in stressed animals suggesting that stress related oxidative damage could have primed apoptotic effects of fluoxetine.

The fluoxetine-induced testicular damage in this study was accompanied by elevation in MDA, which is the product of lipid peroxidation and depletion of GSH. Galecki *et al* [26] concluded that the activity of copper-zinc superoxide dismutase (CuZnSOD) in platelets of depressive patients was lower than in the healthy control group. The activity of CuZnSOD increased after fluoxetine treatment and the concentration of lipid peroxidation (TBARS) level was higher in patients than in the healthy control group. Inkielewicz-Stepniak, [4] reported that chronic fluoxetine treatment increased the levels of TBARS, protein carbonyl groups, and the uric acid content in the liver of rats. Testicular damage observed in this work by fluoxetine was accompanied with reduction in testosterone and LH levels. Rygula *et al* [21] examined the effects of fluoxetine on rats exposed to chronic stress. They found increased adrenal weight and decreased plasma levels of testosterone. Bataineh and Daradka, [6] reported that long-term ingestion of fluoxetine caused a decrease in spermatogenesis and weights of reproductive organs. The hormonal assay also showed significant decrease in testosterone levels and follicle-stimulating hormone (FSH) levels. The observed decrease of testosterone recorded in the present work may have resulted from the direct effect of fluoxetine on Leydig cells. This suppressive effect of fluoxetine on testosterone production may be attributed to the direct effects of reactive oxygen species on testicular steroidogenesis.

Curcumin (diferuloyl methane) is a component of turmeric (*Curcuma longa*), a spice often found in curry powder. In recent years, considerable interest has been focused on curcumin due to its use to treat a wide variety of disorders without any side effects. Curcumin has the potential to treat a wide variety of inflammatory diseases including cancer, diabetes, cardiovascular diseases, arthritis, Alzheimer's disease, psoriasis, *etc*, through modulation of numerous molecular targets [27]. In the present study, treating animals with fluoxetine and curcumin reduced the histopathological changes induced by fluoxetine in testes. Moreover, rats treated with fluoxetine and curcumin exhibited a significant increase in LH and testosterone. These results are in agreement with that of Salama and El-Bahr [28] who reported that the use of curcumin attenuated the damaged effects of cadmium on reproduction of male rats, improved its spermatogenic damage, decreased sperm count, testosterone level and induced antioxidant defense. Ilbey *et al* [29] investigated the effects of curcumin against cisplatin-induced testicular injury in rats. A significant increase in plasma testosterone levels, GSH levels and GSH-Px activity, and a decrease in MDA and NO levels in testicular tissue were observed with cisplatin plus curcumin compared with that with cisplatin alone. Cisplatin caused irregular seminiferous tubules, reduction of seminiferous epithelial layers, significant maturation arrest and perivascular fibrosis. Curcumin administration to cisplatin-treated rats significantly prevented these histopathological changes.

Our results indicated that co-administration of curcumin caused an increase in the antioxidant enzyme, GSH and a decrease in the lipid peroxidation marker, MDA. Similarly, Farombi *et al* [30] indicated that curcumin protected against testicular oxidative damage induced by di-n-butylphthalate. They added that the chemoprotective effect of curcumin was due to its intrinsic antioxidant properties and as such proved useful in combating phthalate-induced reproductive toxicity. Mathuria and Verma [31] reported that curcumin ameliorated aflatoxin-induced lipid peroxidation in liver, kidney and testis of mice. Results of Tirkey *et al* [32] showed that curcumin improved renal GSH levels in cyclosporine-treated rats. Mahfouz *et al* [33] found that curcumin was an effective chain breaking antioxidant which prevented oxidation and lipid modification of low density lipoprotein. The authors suggested that curcumin supplementation could be an effective strategy in preventing low density lipoprotein oxidation and its impact on atherosclerosis and lesion formation. Srinivasan [34] concluded that curcumin inhibited lipid peroxidation by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes, SOD, CAT, glutathione peroxidase and glutathio-s-transferase. Manikandana *et al* [35] reported that curcumin significantly decreased the levels of free radicals and this protective effect was attributed to its free radical scavenging activity, induction of detoxification enzymes and providing protection against degenerative diseases.

In conclusion, the present study showed that curcumin has ameliorative effect on the testicular toxicity of fluoxetine. This may be explained by the fact that it prevents cellular damage occurring as a result of oxidative stress in spermatogenic cells and Leydig cells.

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ADDITIONAL STATEMENTS

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