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TESTICULAR TOXICITY INDUCED BY EXOGENOUS BETAMETHASONE AND THE AMELIORATIVE ROLE OF CURCUMIN IN ADULT AND FETAL STAGES OF ALBINO RATS

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ABSTRACT

Betamethasone is one of the most usual drugs in both human and animal medication. This study dealt with the testicular toxicity induced by exogenous betamethasone in the adult and fetal stages of the albino rats (*Rattus norvegicus*) and its amelioration by curcumin. It involved four parameters, namely, histopathological, histomorphometric, immune-histochemical and biochemical. The results showed that 0.1 mg/kg body weight of betamethasone injected daily to the adult males for 6 days caused destructive changes in the histological architecture and significant decrease in testosterone and luteinizing hormones (LH) concentrations. Male rat fetuses maternally injected with the same dose for the same period (from day 14 to day 19 of pregnancy which constitute the critical days of prenatal reproductive development) also displayed testicular toxicity. In conclusion, the outcome of the present results raise concerns about the use of betamethasone in human therapy. While the results revealed the evident testicular toxicity of betamethasone, it proved that curcumin at a dose of 15.75 mg/kg body weight is a potential ameliorative agent against this toxicity during both adult and fetal stages.

KEYWORDS: Betamethasone, Curcumin, Testicular toxicity, Histopathology, Testosterone, Luteinizing hormone, Fetuses.

INTRODUCTION

Most of chemical drugs have hazard side effects on various organs of the body.[1,2] It is therefore necessary to either identify these effects to better use of drugs or to switch to phytotherapy. Glucocorticoids are strongly immune suppressive and anti-inflammatory; thus, these are among the most commonly drugs prescribed worldwide.[3] They are also one of the most misused medicines and cause numerous side effects on various body systems and it has been reported that the adverse side effects of glucocorticoids are mostly unavoidable and can be only minimized.[4] The seriousness of the glucocorticoids on the embryos is that it can readily cross the placenta and suppress the fetal pituitary adrenocortical axis.[5] Growing concerns have recently been raised regarding the incidence of male reproductive disorders from exposure to endocrine disruptors.[6] Glucocorticoids bring about their multiple effects by activating the intracellular glucocorticoids receptor that binds to specific glucocorticoids-responsive elements in the vicinity of regulated genes and subsequently affect their expression.[7] It is estimated that glucocorticoid receptors can interact as transcription factors for about 30% of genes, so it is not surprising that glucocorticoids induce a wide range of responses.[8,9]

By controlling testosterone secretion via affecting Leydig cells and testosterone making enzymes, the first target for glucocorticoids is the testis.[10] Betamethasone is a corticosteroid which helps lung maturity of fetuses in danger of preterm birth via increase in alveoli surfactants and lung compliance.[11] It is one of the most usual drugs in human and animal medication. In a study for determining various effects of betamethasone on the concentration of male reproductive hormones, it has been reported that betamethasone reduces the concentration of both testosterone and LH hormones in adult mice.[12] It has been reported that

the increased glucocorticoids concentration occurs before a decrease in testosterone concentration.[10]

The importance of medicinal plants as a tool for both treatment and drug supply cannot be denied.[13,14,15] Consequently, a great interest in phytotherapy have been shown in recent years especially those drugs originated from plant. Among the several reasons behind this great interest to plant derived drugs, are the hazard side effects and the difficult access to the conventional drugs in some countries.[1,16] Phytotherapy have been promoted after gradual transition of mono drug therapy in conventional medicine into multi drug therapy.[17] Phytotheraputic agents are safer and more economic than that of synthetic drugs[18] and it has been reported that the way for management of diseases with medicines of more efficiency and less adverse effect is the phytotherapy.[19] Therefore, the use of herbal medicine increases every day and nowadays traditional herbs have more acceptance than prescription drugs in many cultures.

Infertility is one of the major health problems in life, and approximately 30% of infertilities are evidently due to a male factor.[20] The relationship between oxidative stress and infertility has been reported.[21,22] It has therefore become necessary to use fertility-regulating agents of plant origin which are ecofriendly and have fertility enhancing properties.[13,14] Curcumin has recently gained popularity among physicians. A number of studies have investigated the various biological effects of curcumin, attributed to polyphenol's potential to modulate multiple signaling molecules.[23]

There is an urgent need to find fertility enhancing agents from plant origin and curcumin is anticipated to have a great contribution in this regard. However, there are some aspects which need to be consider. One of the important aspects is the accurate dosing. High dose of curcumin (500 - 600 mg per kg body weight caused a reduction in the diameter of seminiferous tubules, loosening of the germinal epithelium, intraperitoneal vacuolation and mixing of spermatids at different stages of spermatogenesis in rats[24,25] and decreased serum testosterone levels in mice at a dose of 500 mg kg body weight.[26] The present study aimed firstly, to investigate the possible hazard effect of betamethasone on the testis of adult and fetal stages of the albino rat. The second aim was to investigate the ability of curcumin to act as an ameliorative agent against this induced toxicity.

MATERIALS AND METHODS

Animals and grouping

All the experiments were done in compliance with the guide for the care and use of laboratory animals approved by Faculty of Science, Menoufiya University, Egypt. Healthy mature virgin females and fertile males of Wistar albino rats (*Rattus norvegicus*), weighing 154 ± 10 g were born and reared at the animal house of Department of Zoology, Faculty of Science, Menoufiya University. They were housed in specially designed plastic rodent cages and maintained at 25 ± 2ºC in 12h light:12h dark cycle. While adult males were provided with rodent pellet and water was available *ad libitium* the females were treated differently. For the latter, Free access of water and standard diet composed of 50% ground, barely, 20% ground yellow maize, 20% milk and 10% vegetables were supplied. Mating was achieved by housing the females with the males at a ratio of one male with two females overnight. Females were checked daily in the morning for the presence of a copulatory plug and the presence of sperms in unstained native vaginal smears. The day at which vaginal smear was positive has been considered as the day zero of pregnancy. The pregnant rats were divided into four groups, four rats each, as follows:

1. Control, administrated distilled water.
2. Curcumin, given oral administration of curcumin (15.75 mg/kg).
3. Betamethasone, given subcutaneous administration of betamethasone (0.1 mg/kg).
4. Betamethasone and curcumin, received subcutaneous administration of betamethasone first followed by oral administration of curcumin one hour later.

For eliminating time differences among all groups, day 20 was determined as the end point for experimentation on the fetal stages. Adult male rats were also divided in the same manner except that the individual group was composed of 8 rats. A total number of 16 females, 32 males, 37 male fetuses were included in the whole investigation. Sexing of the fetuses was performed by observation of the ano-genital distance, which is 1.5 to 2 times greater in males than in females. Female fetuses were discarded.

Betamethasone administration

Betasone tablets (each tablet contains Betamethasone 0.5 mg) was manufactured in Memphis Company for pharmaceutical and chemical industries, Cairo, Egypt and purchased from pharmacy in Shebeen El-Koom, Menoufiya.

The tablets were ground and dissolved in distilled water and subcutaneously administrated for six days at a dose of 0.1mg/kg body weight[27] starting from gestation day (GD) 14 and ending at GD 19 i.e. the critical days of prenatal reproductive development.

Water extraction of curcumin

Dry turmetric rhizomes of the plant *Curcuma longa* were purchased from a local market at Shebeen El-Koom, Menoufiya, Egypt. One-kilogram fresh Curcuma *longa* were crushed into powder, macerated in distilled water, filtered and orally given daily, as mentioned, at a dose of 15.75 mg/kg body weight.[28]

Investigated parameters

A- Histopathological investigation

For light microscopical examination, both adult and fetal individuals were dissected and the right testes were taken out for investigation. Small pieces of adult and the whole fetal testes were fixed by immersion in 10% neutral formalin for 24 hours at room temperature followed by washing under running tap water for 12 hours. All specimens were transferred to 70% ethanol and then dehydrated in an ascending series of ethanol, cleared in xylol and embedded in molten paraffin. Five µm thick sections were produced using a rotary microtome (Leica, Model Rm 2125, Germany). Sections were mounted on albumen-coated slides and stored until staining. Histological staining was performed with Ehrlich's hematoxylin and counterstained with aqueous eosin. The obtained sections were subjected to microscopical examination and when necessarily photographing using Olympus microscope.

B- Histomorphometric measurements

Quantitative measurement was carried out by measuring the diameter of 30 round or nearly round transverse sections of seminiferous tubules. The latter were randomly chosen at 40X magnification and the digitized images were analyzed for morphometric study. The epithelium heights were also measured as mentioned. The linear micrometer was also used to confirm the obtained data.

C- Immuno-histochemical investigation

Avidin-biotin peroxidase method was used for the immuno-histochemical demonstration of the anti-apoptotic mediator Bcl-2 and proapoptotic antigen Caspase-3.[29] The criterion for a positive reaction is a dark, brownish, intracytoplasmic precipitate. For the negative control, the primary antibody was omitted to guard against any false positive results that might develop from a non-specific reaction. Negative control sections were produced by substituting the primary antibodies of Bcl-2 and Caspase-3 by normal goat serum. All stained slides were viewed using Olympus microscope and images were captured by a digital camera (Canon Power Shot A620). Digital images were analyzed by a semi-quantitative scoring system (Figi-Image J software, Java based application for analyzing images).[30]

D- Biochemical investigation

In case of the adult rats, at the end of the experiment and after scarification via decapitation, blood samples were collected from the inferior vena cava of each adult rat for biochemical analysis before dissection and taken the right testes out. After centrifugation of blood samples at 3200 rpm for 30 min, sera were separated and stored at -20°C. A technique using radioimmunoassay kits supplied by Diagnostic Products Corp. (Los Angeles, CA, USA) was employed to measure serum testosterone and luteinizing hormone levels.[31]

E- Data evaluation and statistical analysis

All data sets were expressed as mean ± standard error of the mean (SEM). The statistical data were based on at least 6 testes in each group. The data were analyzed statistically for normal distribution (student's T test) and homogeneity of variances (Levene test) using statistical package of social sciences (SPSS) software for windows, version 22. Differences were considered insignificant whenever $P>0.05$. The significances of the obtained data were classified into three categories according to P values, i.e. $P<0.0001$, $P<0.001$ and $P<0.05$.

RESULTS

I- Histopathological observations

A- Adult testis

Control group

The compactly arranged seminiferous tubules (ST) appeared with normal interstitial spaces (Is) containing interstitial Leydig cells (L). The germinal epithelium was well organized and comprised different stages of the spermatogenic series namely; spermatogonia, spermatocytes, spermatids, and spermatozoa arranged from without inward. The lumen contained abundant amount of spermatids and spermatozoon. The Sertoli cells were found between spermatogenic cells resting on intact basement membrane (Fig. 1 A&B).

Curcumin group

The histological observations of the seminiferous tubules of curcumin group were more or less similar to those of the control. The tubules were condensed with spermatogenic cells, and the lumen was filled with spermatozoon, Spermatogonia were present along the basement membranes and the nuclei were round and compact (Fig. 1 C&D). The cycle of spermatogenesis was regular as in the control group. The same is applied to both the seminiferous tubules' diameter and the epithelial height with no significant difference between the two groups (Fig. 4).

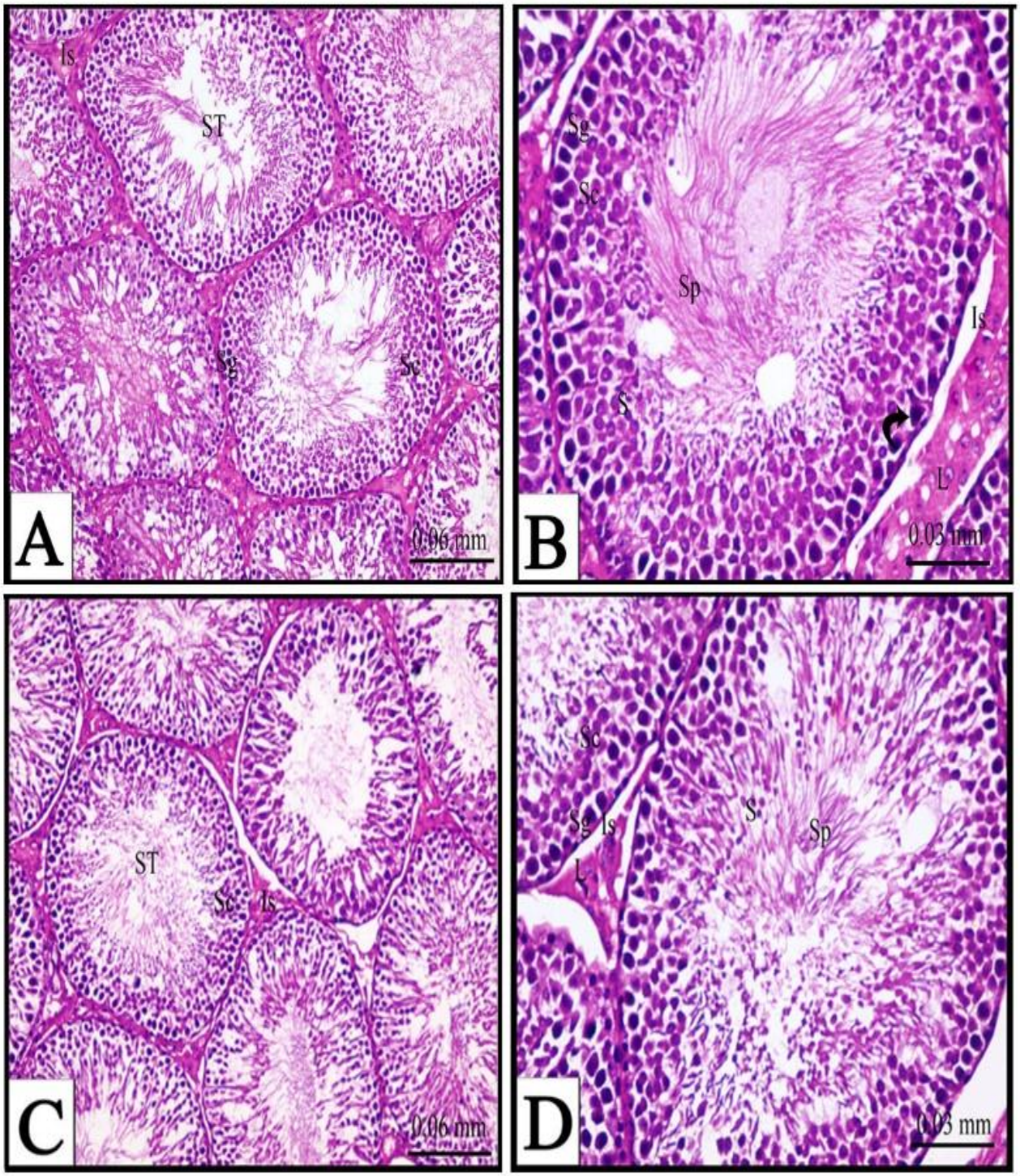

Figure 1: Photomicrographs of transverse sections in the testes of a control (A& B) and curcumin group of adult albino rats showing: normal seminiferous tubules (ST) filled with spermatozoa (Sp), Leyding cells (L) Within the intersticial space (IS). H&E.

Betamethasone group

The ST showed sloughing of germinal epithelium into lumen where the spermatogenesis was highly reduced, the interstitial tissue showed degeneration and the presence of pyknotic nuclei (Fig. 2 A&B). The basement membrane appeared thick, irregular and degenerated. Wide interstitial spaces with atrophied Leydig cells were observed (Figs. 2 B&C). There was evident reduction in size and disorganization of seminiferous tubules which showed marked decrease in spermatogenic cells, and the lumens of some tubules were empty with an evident disturbance in spermatogenesis. Degeneration and atrophy of most seminiferous tubules were seen with loss of spermatogenesis. There was also vascular congestion associated with hemorrhage, edema, marked depletion of the spermatogenic cells in most of the seminiferous tubules as compared with the control group (Figs. 2 E&F).

Seminiferous tubule diameter and germinal epithelium height showed significant decrease compared with the control group (Fig. 4).

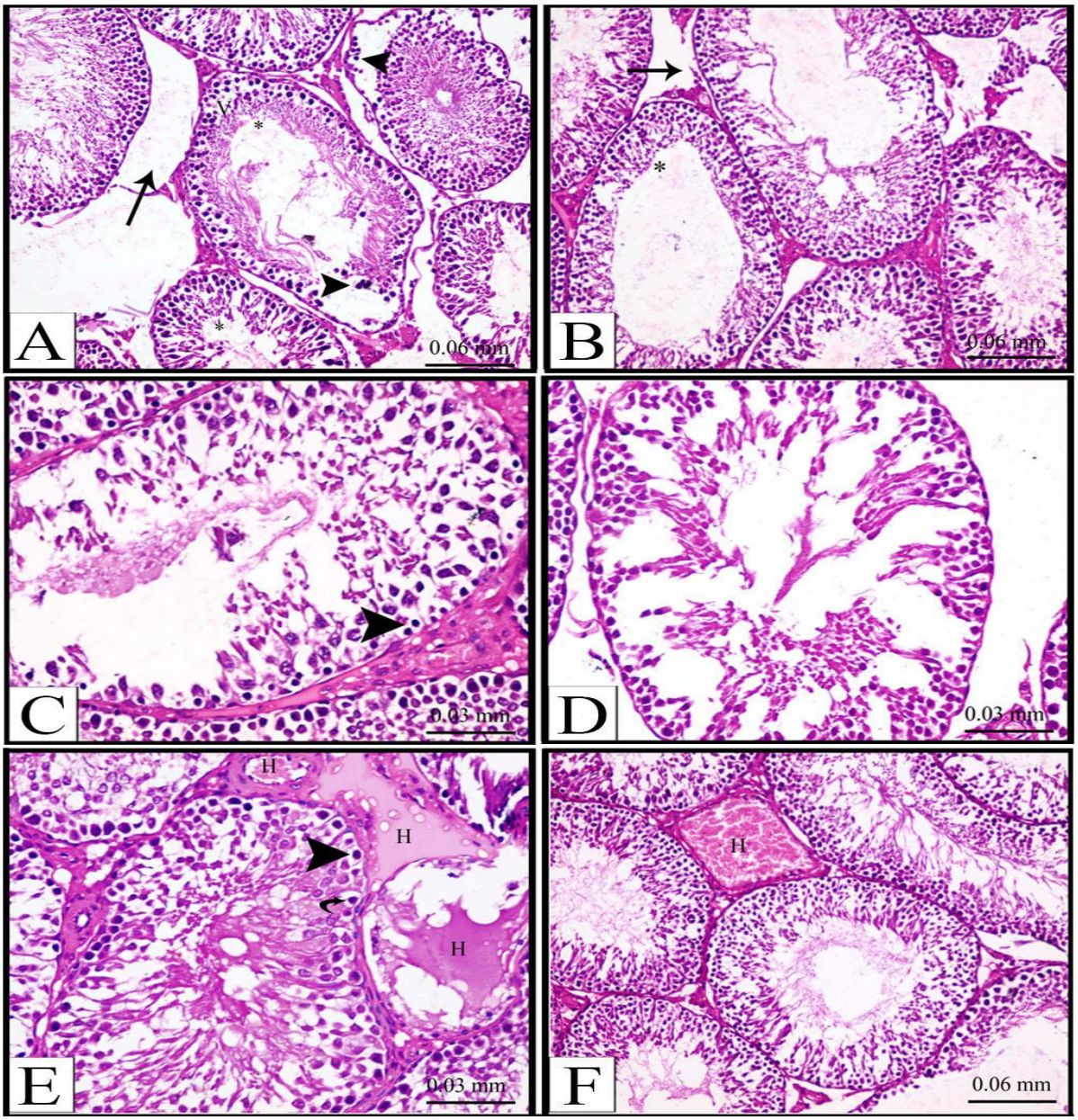

Figure 2: Photomicrographs of transverse sections in the testes of betamethasone group of adult albino rats showing: interstitial tissue (Is), spermatogonia (Sg), seminiferous tubule (ST), spermatocytes (Sc), spermatozoa (Sp), Leyding cell (L), spermatids (S), vacuolation (V), intertubular hemorrhage (H), * refers to reduced spermatogenesis, arrows refer to degenerated interstitial tissue and arrow heads to pyknotic nuclei. H & E.

Betamethasone + curcumin group

The ST appeared with normal germinal epithelium and Sertoli cells were resting on intact basement membrane and spermatozoa bundles appeared inside the lumens. Normal interstitial spaces containing Leydig cells were seen with evident restoration of spermatogenesis (Fig.3 A&B). However, some of the seminiferous tubules had irregular outline. Other seminiferous tubules showed slightly congested blood vessels were observed. Marked improvement in the mean ST diameter and in germ cell height in comparison with betamethasone maternally injected fetuses (Fig. 4).

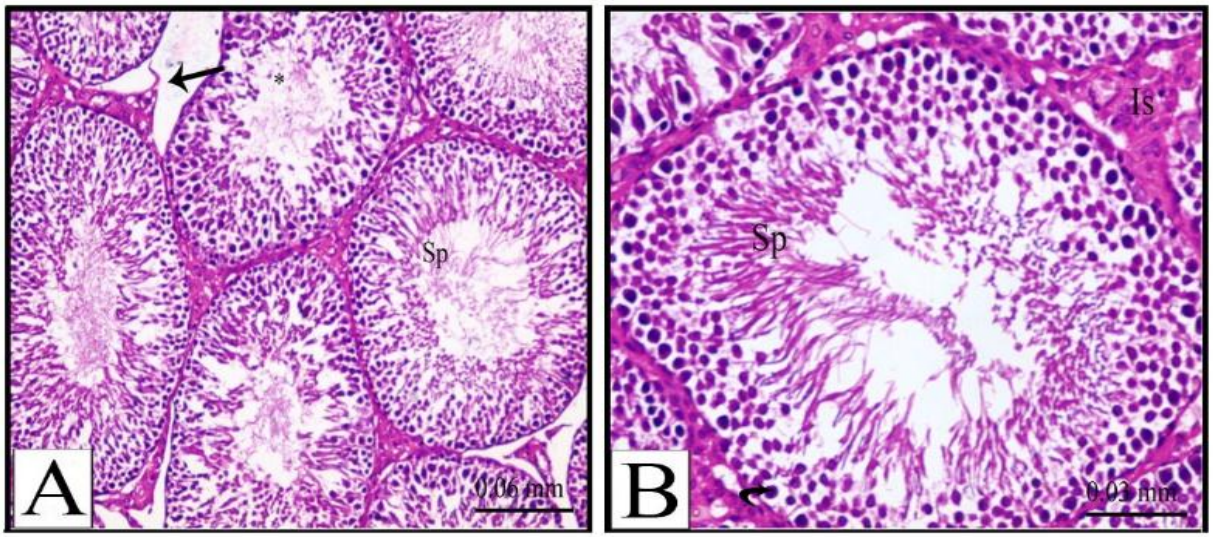

Figure 3: Photomicrographs of transverse sections in the testes of betamethasone and curcumin group of adult albino rats showing nearly normal seminiferous tubules with no or mild congestion H & E.

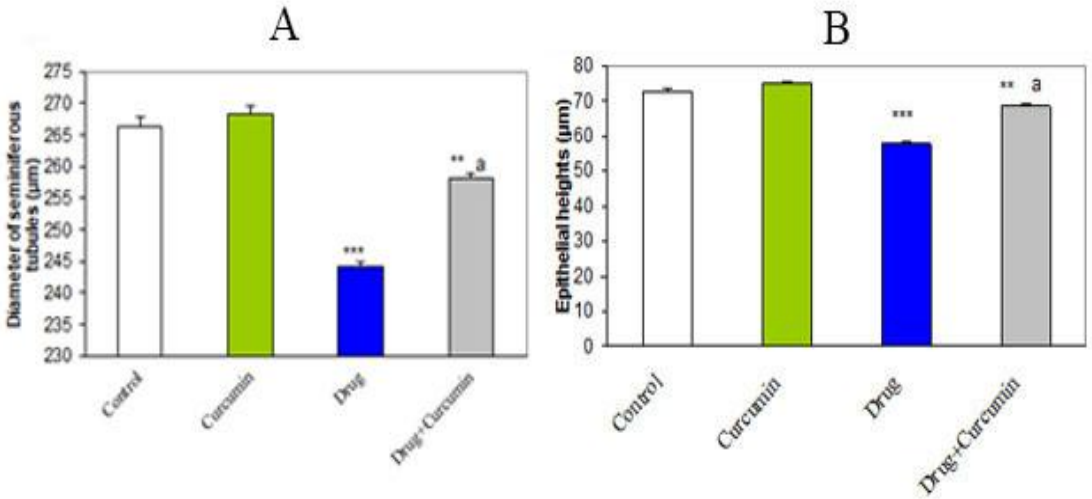

Figure (4): Histogram showing changes in both diameter of seminiferous tubules (A) and epithelial height (B) (mean ± SEM) in different adult groups. Asterisks indicate the statistically significant differences (* P< 0.0001 & ** P< 0.001) versus control.**

a = significant (P<0.001) compared with the betamethasone group.

B- Fetal testis

Control group

As can be observed in Fig. 5 A, the tunica albuginea (TA) completely surrounded the testis and consisted of fibrous tissue. The tunica vasculosa (TV) was well-developed and rich in blood vessels. The seminiferous tubules displayed a notable variability in terms of both size and shape. The interstitial tissue (Is) separated the gathering groups of the seminiferous tubules and contained Leydig cells (L) which were located near to the blood vessels. The basement membrane can easily be identified at high magnification. Most of the Sertoli cells (Se) displayed mitotic division and the gonocytes (g) as

the fetal germ cells were well-defined and constituted the lining of the seminiferous tubules and their numbers within the individual tubule were variable.

Curcumin group

The histological appearance of this group was more or less similar to that of the control group (Fig. 5B). The same was applied to both the seminiferous tubules' diameter and the epithelial height with no significant difference between the two groups (Fig. 6).

Betamethasone group

There were a number of destructive changes. The tunica albuginea was thinner than its control counterpart. The tunica vasculosa contained empty spaces and disrupted connective tissue if compared with both the control and curcumin groups. The seminiferous tubules were disorganized and either far from each other or fused. Some of the Sertoli cells were detached and loosed their normal organization when compared with the control group. There were a number of necrotic areas, pyknotic nuclei and hemorrhage (H) was evident (Fig. 5 C.D&E).

The diameter of the seminiferous tubules and germinal epithelium heights showed significant decrease compared with the control group (Fig. 6).

Curcumin + betamethasone group

The testis of this group displayed a number of ameliorative changes compared with the betamethasone group. The ST appeared more or less similar to that of the control group (Fig. 5F). Marked improvement in the mean ST diameter and in germ cell height in comparison with betamethasone maternally injected fetuses (Fig. 6).

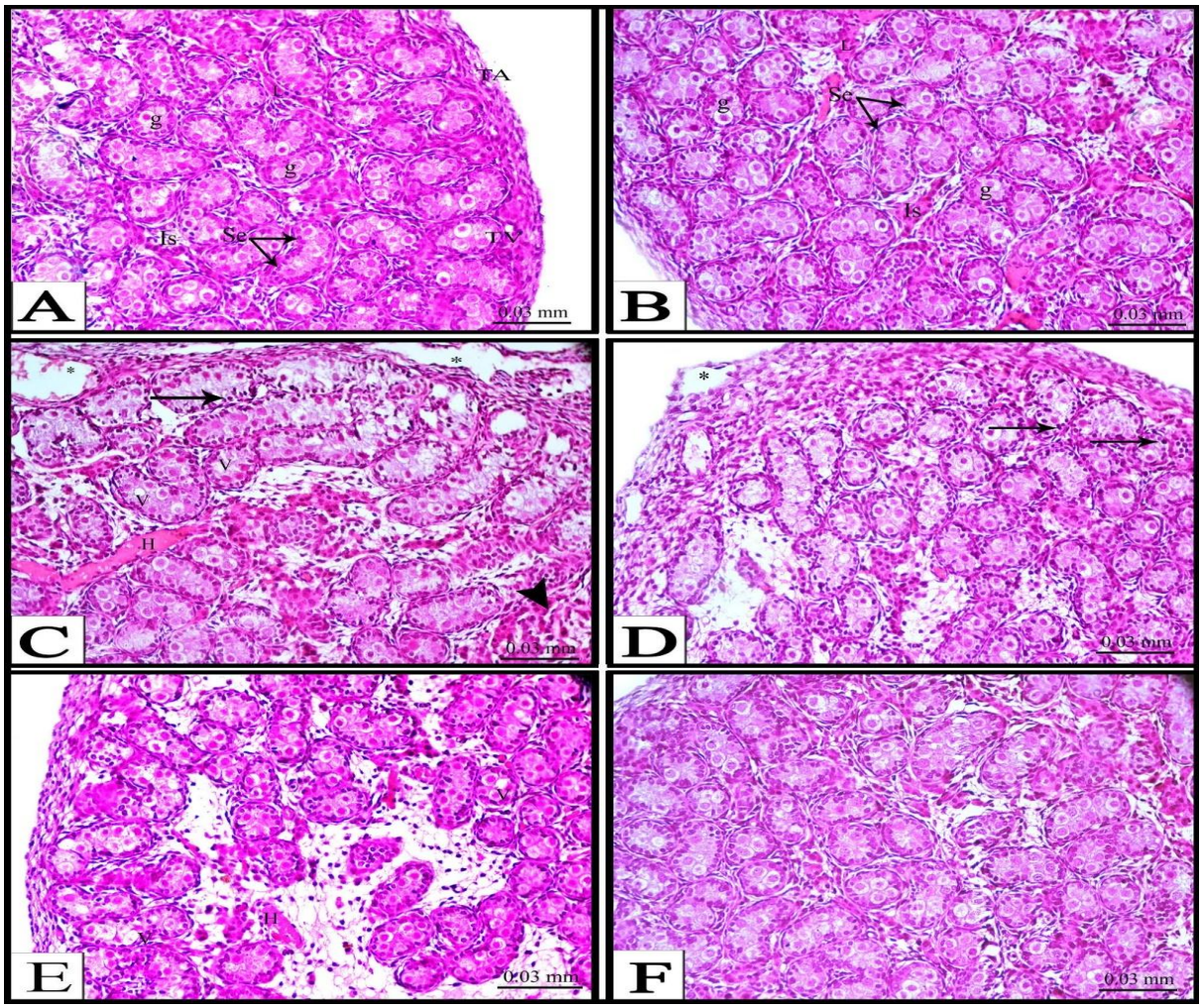

Figure 5: Photomicrographs of transverse sections in the testes of control (A) Curcumin (B), betamethasone (C, D & E) and betamethasone + curcumin (F) groups of 20 days rat fetuses showing (Se Sertoli cell, g gonocytes, L Leyding cell, Is interstitial tissue, H hemorrhage, Arrow pyknotic nuclei, * disruption in capsule, Arrow head necrotic area. H & E.

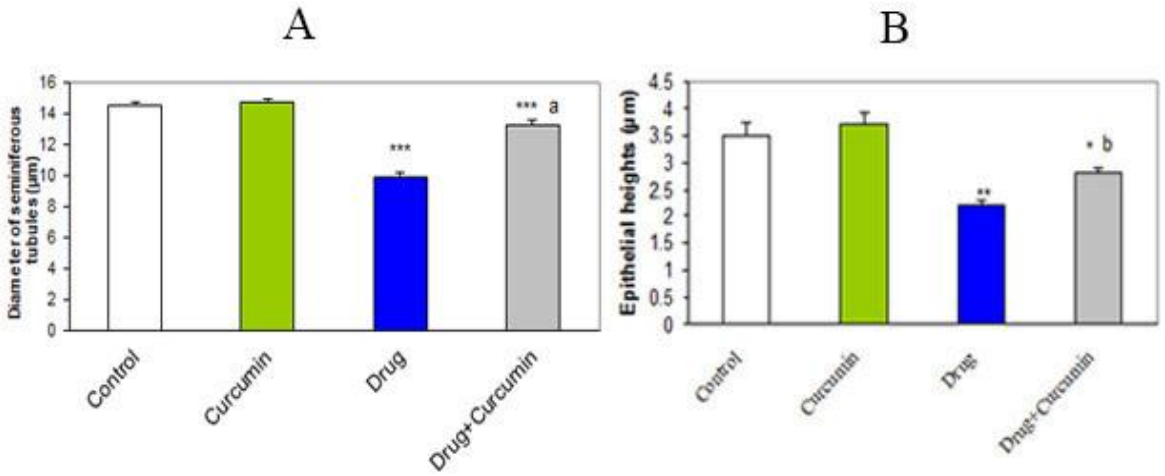

Figure (6): Histogram showing Changes in both diameter of seminiferous tubules (A) and epithelial height (B) (mean ± SEM) in different fetal groups. Asterisks indicate the statistically significant differences (* P< 0.0001 & ** P< 0.001) versus control.**

a = significant (P<0.0001) compared with the betamethasone group.
b = significant (P<0.05) compared with the betamethasone group.

II- Immuno-histochemical observation

A- Adult testis

As shown in Figs. 7,8 & 9 and Table 1, testicular tissues from control (28.94 ± 0.25) and curcumin (27.01 ± 0.25) groups stained strongly for Bcl-2 while little staining was observed for Caspase-3 (8.87 ± 0.23 and 10.36 ± 0.33 respectively). In contrast, testicular tissues from rats with betamethasone alone stained strongly for Caspase 3 (32.46 ± 0.39) but only weakly for Bcl-2 (5.78 ± 0.21). Moderate immune-reactivity was observed in the combined group (15.76 ± 0.29 and 18.80 ± 0.25 for Bcl-2 and Caspase-3 respectively.

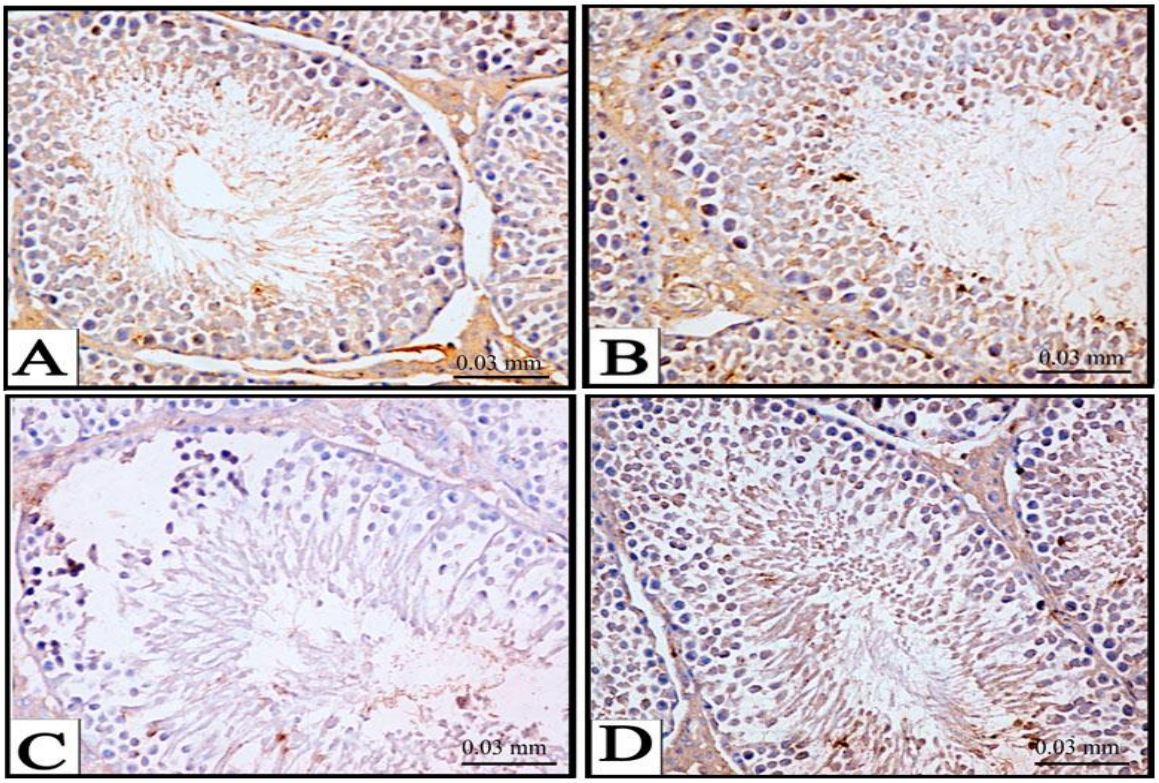

Figure (7): Photomicrographs showing immuno-histochemical localization of Bcl-2 antigen in the adult testis of different groups. A Control, B Curcumin, C Betamethasone and D Betamethasone + Curcumin.

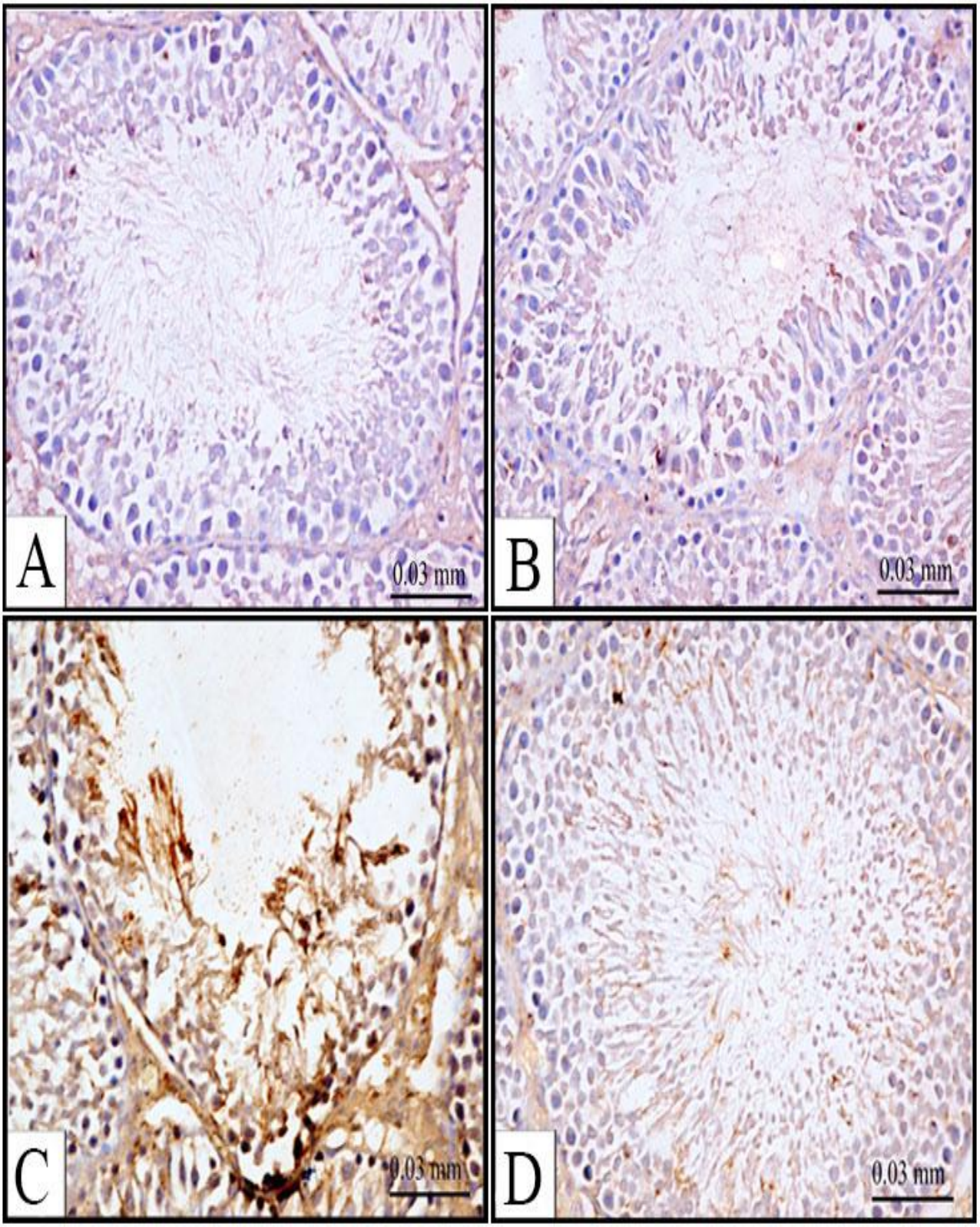

Figure (8): Photomicrographs showing immuno-histochemical localization of Caspase-3 antigen in the adult testis of different groups. A Control, B Curcumin, C Betamethasone and D Betamethasone + Curcumin.

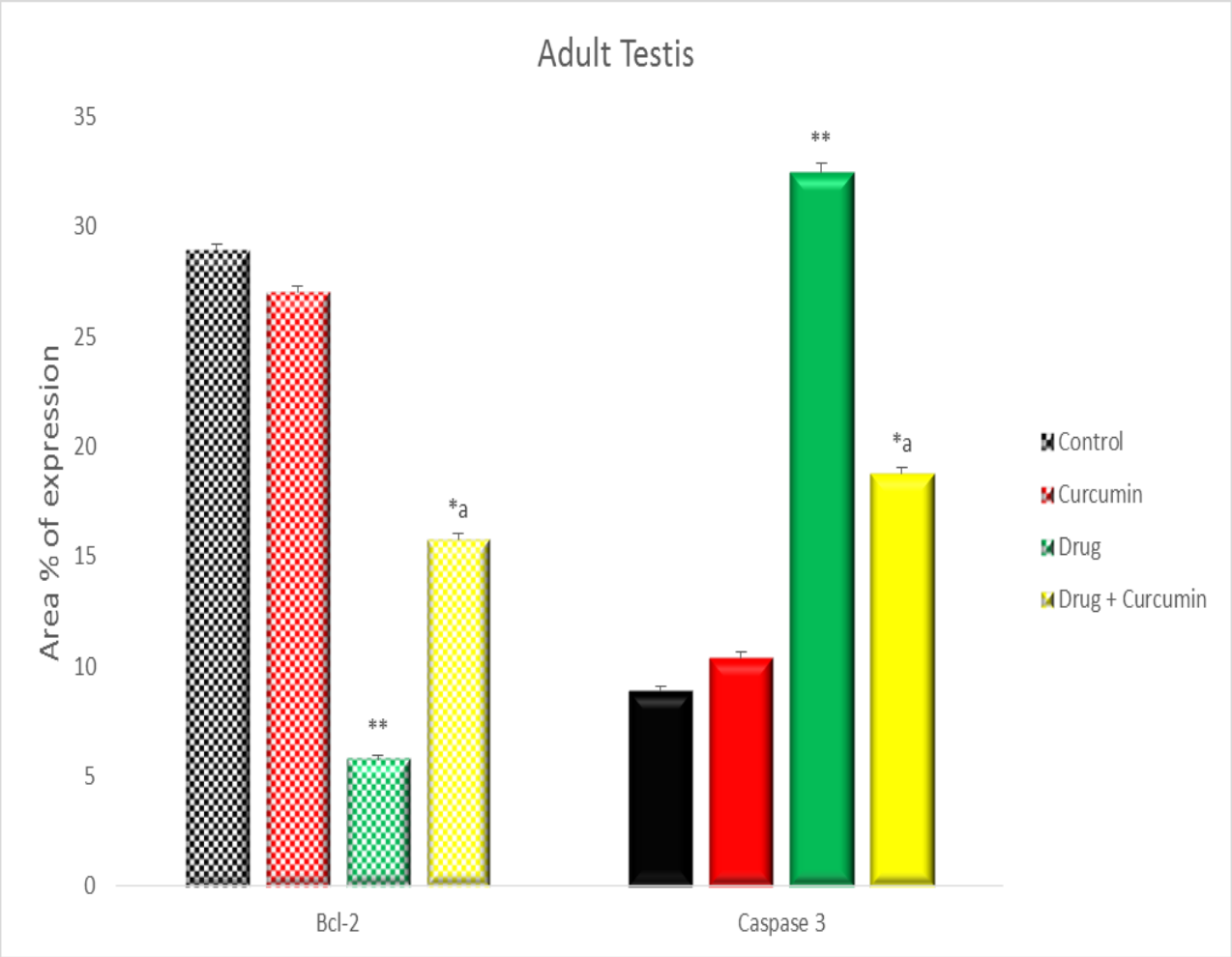

Figure (9): Histogram showing percentages of Bcl-2 and Caspase-3 expression (mean ± SEM) in the testis of control and experimental adult groups. Asterisks indicate the statistically significant differences (P< 0.001 & * P< 0.05) versus control.**
a = significant (P<0.05) compared with the betamethasone group.

Table 1: The mean area % of Bcl-2 and Caspase-3 expression in the adult testis of all groups.

Groups	Control	Curcumin	Drug	Drug + Curcumin
Bcl-2	28.94 ± 0.25	27.01 ± 0.25	5.78 ± 0.21**	15.76 ± 0.29*a
Caspase-3	8.87 ± 0.23	10.36 ± 0.33	32.46 ± 0.39**	18.80 ± 0.25*a

Data are represented as mean area % ± SEM.
Asterisks (* - **) refer to the P value compared with the control group.
a= significant (P<0.05) compared with betamethasone group.
**** P< 0.001 * P< 0.05**

B- Fetal testis

Figs. 10,11 & 12 and Table 2 demonstrate the fetal immune-reactivity to Bcl2 and Caspace-3. Testicular tissues from control (30.57 ± 0.42) and curcumin (29.03 ± 0.35) groups stained strongly for Bcl-2 while little staining was observed for Caspase-3 (9.07 ± 0.23 and 11.13 ± 0.22 respectively). In contrast, testicular tissues from rats with betamethasone alone stained strongly for Caspase 3 (26.37 ± 0.32) but only weakly for Bcl-2 (8.47 ± 0.18). Moderate immune-reactivity was observed in the combined group (18.92 ± 0.22 and 16.05 ± 0.25 for Bcl-2 and Caspase-3 respectively.

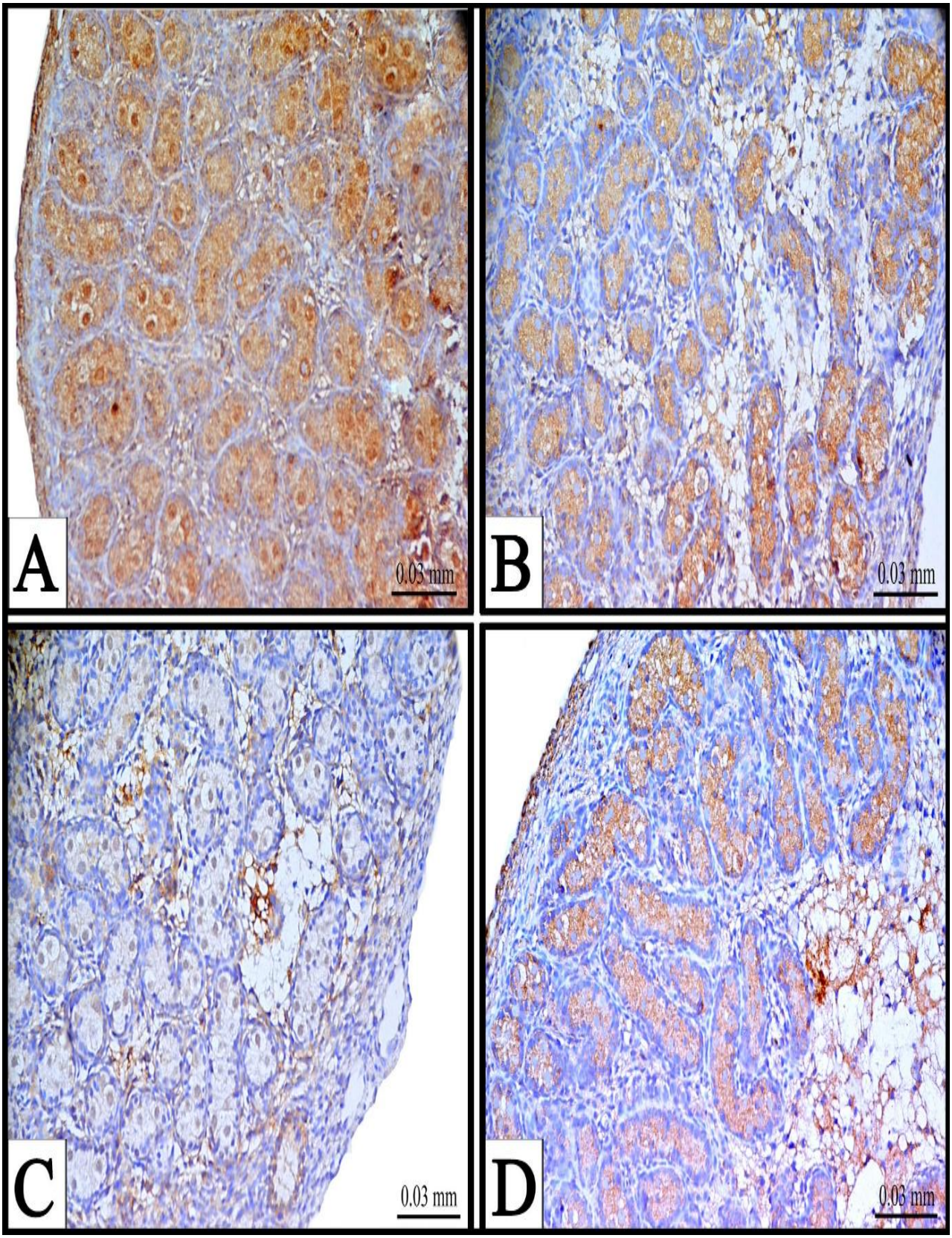

Figure (10): Photomicrographs showing immuno-histochemical localization of Bcl-2 antigen in the testis of 20-day old rat fetuses in different groups. A Control, B Curcumin, C Betamethasone and D Betamethasone + Curcumin.

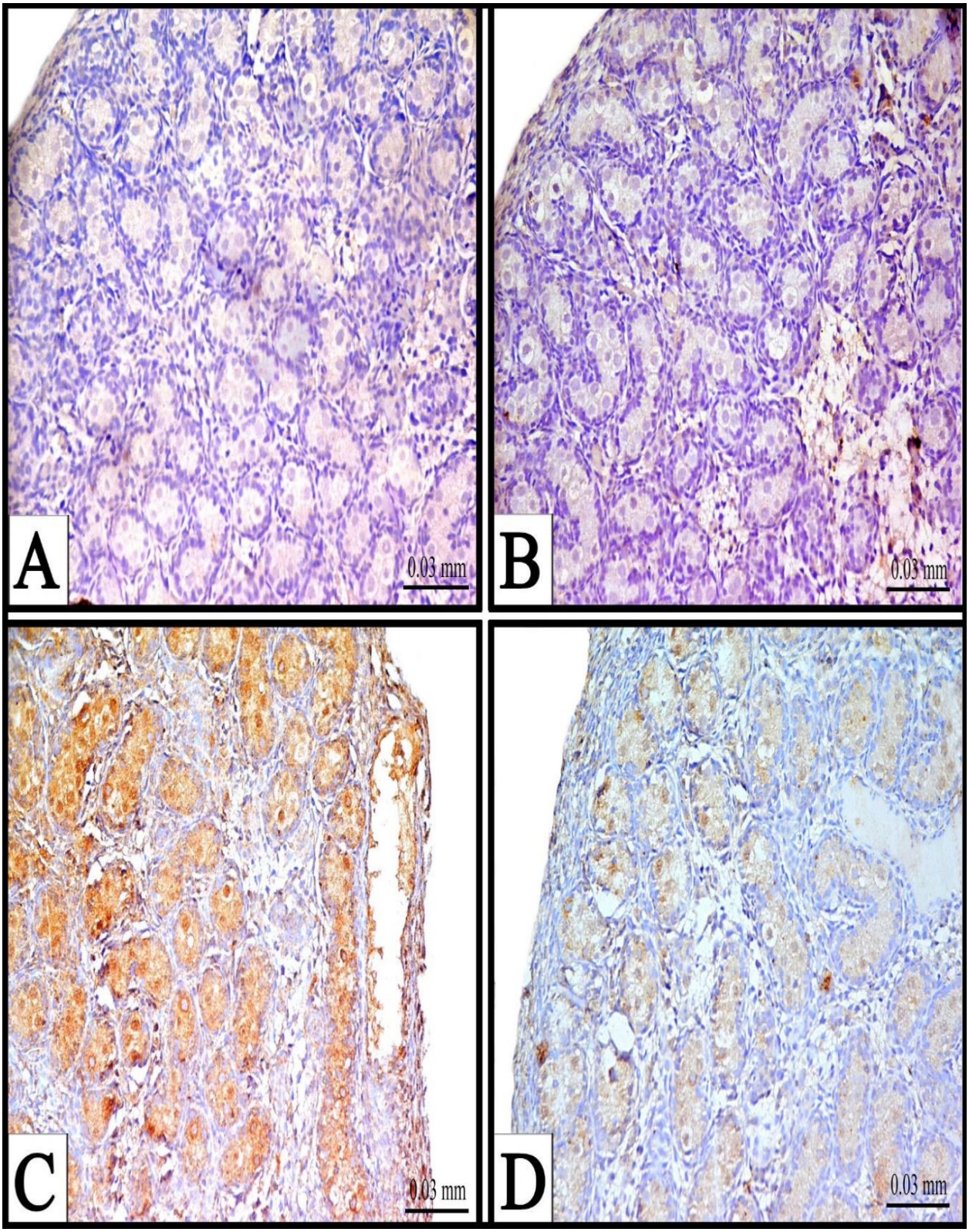

Figure (11): Photomicrographs showing immuno-histochemical localization of Caspase-3 antigen in the testis of rat fetuses in different groups. A Control, B Curcumin, C Betamethasone and D Betamethasone + Curcumin.

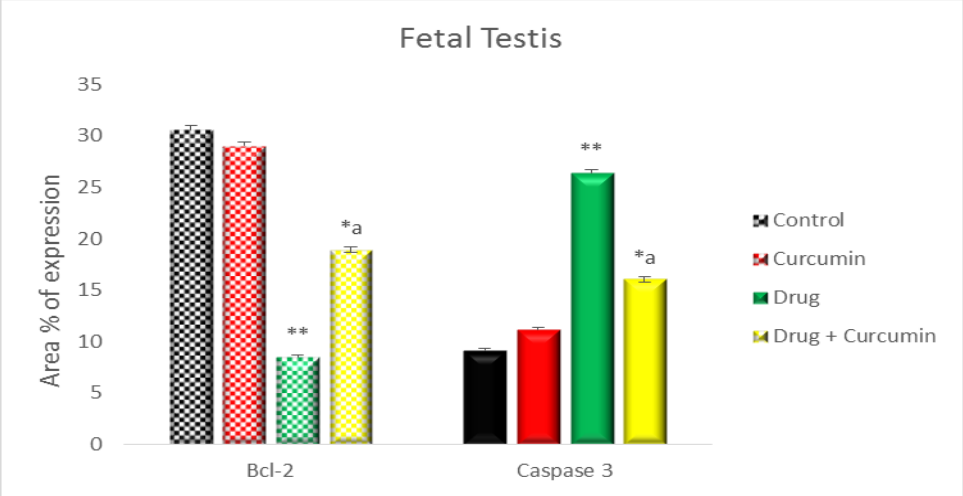

Figure (12): Histogram showing percentages of Bcl-2 and Caspase-3 expression (mean ± SEM) in the testis of control and experimental rat fetuses. Asterisks indicate the statistically significant differences (P< 0.001 & * P< 0.05) versus control.**
a = significant (P<0.05) compared with the betamethasone group.

Table 2: The mean area % of Bcl-2 and Caspase-3 expression in the fetal testis of all groups.

Groups	Control	Curcumin	Drug	Drug + Curcumin
Bcl-2	30.57 ± 0.42	29.03 ± 0.35	8.47 ± 0.18**	18.92 ± 0.22*a
Caspase-3	9.07 ± 0.23	11.13 ± 0.22	26.37 ± 0.32**	16.05 ± 0.25*a

Data are represented as mean area % ± SEM.
Asterisks (* - **) refer to the P value compared with the control group.
a= significant (P<0.05) compared with betamethasone group.
**** P< 0.001 * P< 0.05**

III- Biochemical observations
Figure 13 demonstrates the changes in the testosterone and LH levels in the adult testis of control and experimental groups. Testosterone levels of the curcumin group were above the control level, but no statistical difference was observed. Both testosterone and LH levels decreased significantly in the betamethasone injected adult rats as compared with control (P< 0.0001). Plasma levels of testosterone were significantly increased in betamethasone and curcumin group compared with the betamethasone group (P<0.0001). However, less significance was recorded for the plasma levels of LH (P<0.001).

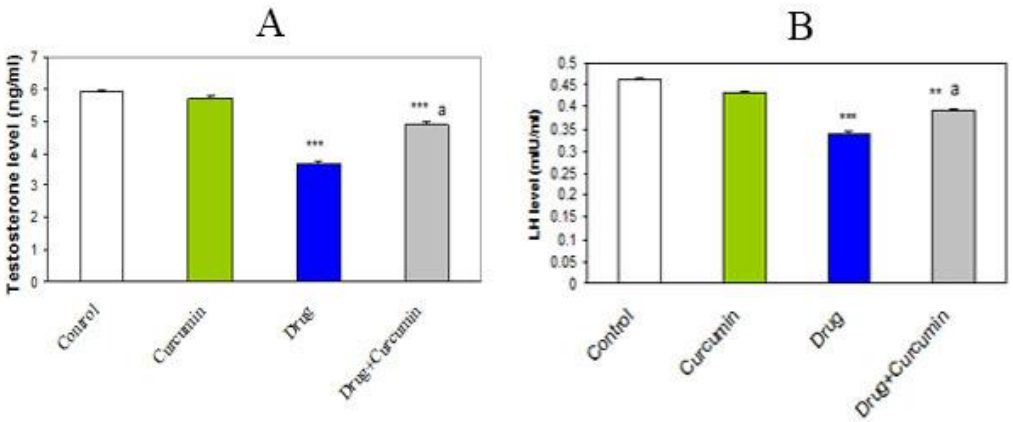

Figure (13): Histogram showing changes in the testosterone (A) and LH (B) levels (mean ± SEM) in the testis of control and experimental groups. Asterisks indicate the statistically significant differences (* P< 0.0001 & ** P< 0.001) versus control.**
a = significant (P<0.05) compared with the betamethasone group.

DISCUSSION

The data presented in this study clearly demonstrate that exposure of adult male rats to betamethasone at a dose of 0.1mg/kg body weight for a period of 6 days resulted in evident testicular toxicity. The latter was expressed histologically, immune-histochemically and biochemically. The fetal stage also reflected testicular toxicity after maternally injected with the same dose for the same period. With the administration of curcumin, the structural changes were ameliorated and ST regained their normal germinal epithelium and Sertoli cells with intact basement membrane and presence of sperm bundles inside their lumens. These findings are in agreement with the previous studies on curcumin as protective against other toxicants.[32,33,34,35] The ameliorating effect of curcumin against the testicular toxicity induced by betamethasone provides evidence for reversibility of this toxicity. The ameliorating effect of curcumin observed in this study is not surprising as it has been previously reported that curcumin reduces reproductive toxicity and therefore could provide a viable food based approach for enhancing male fertility.[35,36,37,38] Curcumin was found to be an important antioxidant and can be effective in the protection of induced reproductive toxicity.[39] Curcumin was found to be useful for prevention of cadmium induced reproductive damage.[33] In another study using guinea pigs as an experimental model Fetouh and Azab[34] demonstrated that curcumin has an ameliorating effects against gentamicin-induced reproductive toxicity.

The reason of terminating the experiments on day 20^{th} is based on the fact that glucocorticoids when injected during organogenesis resulted in one-day delay of parturition compared with control[4,27] and own observation).

The diameter of ST has a significance in that there is a positive relation between tubular diameter and spermatogenetic activity of testis.[20] The diameter in betamethasone injected animals were reduced and the differences were significant compared to control group. The smaller seminiferous tubule diameter may be attributed to the thinning seminiferous germinal epithelium. With co-administration of curcumin these parameters significantly ameliorated as compared to betamethasone injected adult and maternally injected fetuses.

Tissue development involves both cell proliferation and cell death. In sheep fetuses, Pedrana et al.,[40] suggested that a cell death pathway involves changes in the balance of the apoptosis enzymes, caspase-3 and Bcl-2, is activated within the testis by glucocorticoid administration during the last trimester of gestation. The present study provided evidence for betamethasone induced apoptosis of adult and fetal testicular cells of rat fetuses. Bcl-2 expression was very low in the cytoplasm of the testicular tissue of the betamethasone group in both stages, while it was highly expressed in the control group. On the contrary, Caspase-3 expression levels were elevated in the betamethasone group and severely decreased in the control group. These results go in hand with the study of Gouyandeh et al.,[41] who reported that betamethasone induces apoptosis of testicular cells and reduces both spermatogenesis and testosterone level in adult male mice causes reduced fertility and therefore have adverse effects on male reproduction. In a recent study, Sarhan,[42] proved that the long term consumption of monosodium glutamate induces biochemical, structural, and ultrastructural alterations in the rat testicular tissue through induction of oxidative stress which reflects negatively on male fertility. The latter study utilised sodium selenite to ameliorate the toxic effects of monosodium glutamate by reducing the oxidative damage and apoptosis through decreased expression of caspase-3.

Similarly, Pedrana et al.,[40] observed an elevated expression for caspase-3 in sheep during pre- and post-natal development after glucocorticoid administration. The immuno-histochemical investigation of the present study revealed that curcumin leads to suppression of apoptosis evidenced by increased expression of Bcl-2 and decreased expression of Caspase-3 in the combined betamethasone and curcumin group compared with the betamethasone only. This was consistent with the study of Pedrana et al.,[40] who stated that the destructive histological changes were accompanied by changes in the balance between apoptosis-inhibiting and apoptosis-inducing proteins as the testis develops, suggesting that the glucocorticoids have immediate and long-term effects on the processes that modify tissue structure during the development of spermatogenic function. According to the results of this study, it seems that betamethasone reduces testicular function through different mechanisms. It is probably able to directly

affect the activity of testis spermatogenesis or indirectly via acting on protein expression causing apoptosis to the germ cells via interstice pathway.[8,22] High levels of prenatally administered glucocorticoid in sheep reduce fetal testicular development, perhaps via changes in the balance between pro- and anti-apoptotic proteins and cell-cycle proteins.[43] In the adult, stress-induced endogenous glucocorticoids and exogenous glucocorticoids can also initiate apoptosis in Leydig cells thus reducing steroidogenesis and increasing the rate of apoptosis in germ cells.[7,44]

Testosterone is needed to initiate spermatogenesis at puberty and for maintenance of this process in adult. It is also, required for completion of meiosis and for differentiation of spermatids.[23] Testosterone is secreted by the interstitial cells of Leydig in the testis, but only when they are stimulated by LH from the anterior pituitary gland. It has been reported that the quantity of testosterone secreted increases approximately in direct proportion to the amount of LH available.[12] Increased serum concentrations of glucocorticoids induced by stress decreased Leydig cell activity and therefore reduced testosterone production.[22,45] It has been also reported that prenatal dexamethasone exposure perturbed Leydig cell and therefore decrease testosterone production in both pubertal and adult rats.[26] According to the present study, betamethasone administration caused significant reduction in both testosterone and LH levels in adult albino rats. The reduction in the testosterone levels in the betamethasone group of this study is possibly due to a direct action of betamethasone on Leydig cells which are responsible for testosterone secretion. This speculation was based on the depletion of Leydig cells in the interstitial tissue. Indeed, Castro et al.,[46] reported a significant correlation between number of Leydig cells per gram of testis to plasma testosterone level.

Testosterone and LH levels are very much correlate to the histological changes in the tests which have shown decrease in the diameter of seminiferous tubules, widening of interstitial spaces with an evident decrease in the number of Leydig cells. The biochemical changes in the concentration of testosterone and LH were confirmed by histopathological examinations showing severe destruction of seminiferous tubules. It has been speculated that intrauterine betamethasone administration appears to promote reproductive programming and impairment of rat sexual development and fertility due to, at least in part, unusual testicular disorders.[47,48]

It can be concluded that betamethasone injection for 6 days produced destructive effects on the structure of both adult and fetal rat testes. Consequently, the use of betamethasone must be treated with great caution. The safety concerns based on several investigations that has been conducted in our lab call for banning this drug especially during pregnancy. Furthermore, curcumin can be a potential candidate agent against the testicular toxicity induced by betamethasone, possibly via its antioxidant and free radical-scavenging properties.[49] However, further investigations are needed to demonstrate the exact mechanism of curcumin action on betamethasone induced reproductive toxicity.

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