Curcumin Possible Protective Role in Acute Adriamycin Testicular Toxicity in Adult Male Albino Rats (Histological, Histochemical and Immunohistochemical Study)

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Abstract: Introduction: Adriamycin is a commonly used anti-neoplastic agent in the treatment of a wide range of cancers, including hematological malignancies , many types of carcinoma as (bladder, breast, stomach, lung, ovaries). Clinical and experimental studies have widely demonstrated the testicular as well as other organ toxicity caused by adriamycin. **Aim of the work:** This work aimed to clarify toxic effects of adriamycin on the rat testis and the possible protective role of curcumin. **Material and Methods:** This study was carried out on twenty-four adult male Albino rats that divided randomly into four equal groups: control; curcumin treated; Adriamycin treated and protective (Adriamycin and curcumin treated). Curcumin was administered orally to rats at a dose of (200 mg/kg b.wt./day) for nine days. Adriamycin was administered intraperitoneally to the animals at a dose of (25 mg/kg b.wt.) on day seven .all animals were sacrificed on day nine. Testis of each animal was processed for histological, histochemical and immunhistochemial studies. **Results:** Histologically and histochemically, testis of adriamycin treated rats showed pathological changes in the form of exfoliation, disorganization and degeneration of spermatogenic cells and appearance of vacuoles replacing spermatogenic cells & dilated interstitium. There was increase of PAS reaction in basal laminae, sperms, spermatogonia and interstitium. On the other hand, there was mild Feulgen reaction in the nuclei of spermatogonia and weak reaction in nuclei of other spermatogenic cells. Immunohistochemically, all cytoplasm of spermatogenic cell layers of seminefrous tubules of treated group showed positive immunoreactivity for caspus III in the form of intense brown reaction. On the other hand, histological, histochemical and immunohistochemical examination of the prophylactic group displayed normal appearance of most spermatogenic cells in seminefrous tubules, but still some tubules appeared distorted. **Conclusion:** toxic effect of adriamycin should be kept in mind during chemotherapeutic treatment . Curcumin advised to be administered in concomitant with adriamycin treatment as it could ameliorate adriamycin toxicity on testis.
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Key Words: Adriamycin – Curcumin- Chemotherapeutic – Rat testis

1. Introduction

Adriamycin is a commonly used anti-neoplastic agent in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma (bladder, breast, stomach, lung, ovaries, thyroid) and soft tissue sarcomas (**Laginha , 2007**).

Although adriamycin is considered a very potent and efficient chemotherapeutic drug, it also kills healthy cells, especially those under rapid and constant proliferation, such as the male germ cells. Clinical and experimental studies have widely demonstrated the testicular toxicity caused by doxorubicin (**Damani *et al.,* 2002**). **Lu and Meistrich (1979)** showed that, even a low dose of doxorubicin (1 mg/kg b.w.) given to adult mice is able to target germ cells, mainly A1-A4 spermatogonia, leading to seminiferous epithelium depletion. It has been shown that, adriamycin causes germ cell apoptosis (**Suominen *et al.,* 2003;Hou *et al.,* 2005; Yeh *et al.,* 2007**), reduction of sperm counts (**Kato *et al.,* 2001; Yeh *et al.,* 2007**) and decrease in testicular weight (**Yeh *et al.,* 2007**). Adriamycin also affects testicular lipids (**Zanetti *et al.,* 2007**).

Adriamycin has multiple mechanisms of action, the main target of adriamycin is the DNA of dividing cells; One mechanism of adriamycin cytotoxicity is intercalation in the DNA. Once the drug penetrates into the cell, it binds to chromatin. It then forms a complex with the DNA by intercalation of its planar rings between nucleotide base pairs causing cell cycle blockage in the G2 phase, single-strand breaks (**Kiyomiya *et al.,* 2001; Yokochi and Robertson, 2004**) and inhibition of the activity of some nuclear proteins, such as DNA and RNA-polimerase and DNA-topoisomerase II (**Speth *et al.,* 1988; Fimognari *et al.,* 2008**). Moreover, **Xu *et al.,* (2005)** referred that, adriamycin interact with metal ion chelation and free radical generation.

Based on this concept, a variety of anti-oxidant or anti-apoptotic agents have been employed to counteract adriamycin induced testicular damage

(**Hou *et al.,* 2005**). Nevertheless, there is still no single agent proven to be effective enough to prevent or reverse this adverse effect (**Vendramini *et al.,* 2010**).

Curcumin (CMN), a yellow pigment present in the rhizome of turmeric (*Curcuma longa* L. *Zingiber acesae*), has a wide spectrum of pharmacological and biological activities (**Arau´jo and Leon, 2001**). CMN has been suggested to be a potential anti-inflammatory agent (**Arau´jo and Leon, 2001; Sharma *et al.,* 2007**) and antioxidant (**Balasubramanyam *et al.,* 2003; Priyadarsini *et al.,* 2003**) with phytonutrient and bioprotective properties. CMN exerts anti-inflammatory and growth inhibitory effects in TNF-a-treated HaCaT cells through inhibition of NF-kB and MAPK pathways (**Cho *et al.,* 2007**). Many researchers extensively investigated the chemo-protective properties of CMN and claimed to be linked to its anti-inflammatory (**Nonn *et al.,* 2007**) and antioxidant activities (**Piper *et al.,* 1998**).

The aim of the present study is therefore to assess the possible protective effect of curcumin with single dose doxorubicin induced testicular germ cell toxic model using simple histological histochemical & 92 mmune-histochemical methods.

2. Material and methods:

Matrials :

Animals:

Twenty-four adult male Albino rats of an average weight (150-200 grams) were selected for this study. The animals obtained from breeding animal house, Faculty of Medicine, Menofia University (Menofia, Egypt). During the study, they were feed standard animal food and tap water *ad libitum*. They also, kept under good hygienic conditions and maintained at normal room temperature.

Drugs and chemicals:

Doxorubcin hydrochloride (adricin) :

Vial contained 50 mg / 25 ml obtained from EIMC United Pharmaceuticals Company, Cairo - Egypt.

Curcumin: powder, 200 mg/kg b.wt.

Experimental protocol:

Animal experimentations were carried out in an ethical manner following guidelines set by Ethical Committee of Menofia University. The animals were divided into 4 groups, each of 6 rats: **Group I**: Rats in this group were given the same amount of vehicle (distilled water) orally by intragastric gavage along the time of the experiment (nine days). **Group II**: Rats in this group were orally administered curcumin (200 mg/kg b.wt./ day) orally for nine days as protective dose (**Vankatesan., 1998**). Curcumin was suspended in distilled water for oral administration. **Group III**: Rats in this group were treated intraperitoneally with single dose (25 mg/kg b.wt.) of doxorubicin and were sacrificed after 48 hrs (**Iqbal *et al.,* 2008**) .This doses was reported to be toxic for rats. **Group IV**: Rats in this group were received curcumin (200 mg/kg b.wt./day) orally daily for 9 days. On day seven, one hour after curcumin administration; rats were injected intraperitoneally with single dose (25 mg/kg b.wt.) of doxorubicin. On day nine, 48 hrs after drug injection, the rats were sacrificed. From each rat, both testes were weighted with an electronic balance in gm. The mean weight of testis was calculated and expressed as mean± SD. Statistical analysis was done using student t. test to compare testis weight between different groups, significant value $P< 0.05$. Specimen of right testis were excised then immersed in normal saline. The tissues were divided and subjected to the following studies:

I Histopathological **evaluation:**

The right testis of each animal was dissected out then fixed in 10% formal saline. The specimens were processed to obtain paraffin blocks from which 5 µm thick sections were cut and stained with: haematoxylin & eosin (**Stevens and Wilson, 1996**).

II- Histochemical studies:

Paraffin sections were stained with PAS stain & Fulgen reaction. PAS stain is specific for estimation of glycogen. PAS positive reaction appeared magenta red (**Stevens and Wilson, 1996**).

Detection of apoptosis:

Apoptosis was detected in histological sections by using the Fuelgen reaction. It is specific for staining of DNA of cells. The sections were dewaxed with xylene and hydrated with descending grades of ethyl alcohol and placed in distilled water for 5 minutes. The sections were placed in Schiff's reagent for 45 minutes. It was then rinsed in bisulphite solution (10% potassium metabisulphite) followed by wash in distilled water. Counter staining of the sections were done with 1% light green. The slides were dehydrated with ascending grades of alcohol, cleared with xylene and finally mounted with DPX mounting media. The slides were observed under light microscope for detection of apoptotic cells (Fulgen positive) which stain magenta red in color (**Bancroft and Stevans, 1996**).

III-Immuno-histochemical study:

For this study, paraffin sections 4 µm thick were mounted on glass slides coated on pol-L-lysine, deparaffinized sections were dehydrated and then immersed in 10-3 M sodium citrate buffer (pH 6.0). Sections were then heated in a microwave oven at 60°C for 10 minutes. Endogenous peroxidase was

inactivated by incubating sections with 3% hydrogen peroxide, and nonspecific reactions were blocked by incubating sections in a solution containing 5% normal horse serum and 1% normal goat serum. Then sections were incubated with the primary antibody overnight at 4 °C. Activated caspase-3 expression was assessed using a peroxidase-conjugated rabbit monoclonal antibody IgG (Cell Signaling Technology, Ipswich, MA) (dilution1:200). After 3 rinses with phosphate-buffered saline, the sections were incubated with a commercial kit (Pic-TureTM, Zymed, and South San Francisco, CA) for visualization of the immunoreaction. Finally, they were rinsed with distilled water and counterstained with Mayer's hematoxylin. Normal lymphoid tissue was used as positive control. Negative control was performed by omitting the primary antibody step consequently no immune-staining occurred (**Lee *et al.*, 2006**).

3.Results

Assessment of the testis weight:

Administration of adriamycin alone significantly reduced testis weight as compared to control group ($p< 0.01$). Administration of curcumin in concomitant with adriamycin restorted testis weight to normal (Table.1).

Table 1: Effect of administration of adriamycin alone and in concomitant with curcumin on rat testis weight.

Groups	Weight of testis in gm
Control (Group I)	1.9 ± 0.09
Curcumin (Group II)	1.8 ± 0.08
Adriamycin (Group III)	1.4 ± 0.075**
Adriamycin & curcumin (Group IV)	1.56 ± 0.082 **NS**

Values are expressed as mean ± SD. Group III was compared with group I. Group III was compared with group IV. *$P< 0.05$, **$P < 0.01$, ***$P< 0.001$, NS: Non significant.

Group I: Control group :

Examination of sections of the right testis of control adult male albino rats showed seminifrous tubules. Each tubule is bounded by a basal lamina and lined by spermatogenic and sertoli cells. Spermatogenic cells were arranged in layers occupying space between basement membrane and lumen of the tubule including; spermatogonia, spermatocytes, spermatids and spermatozoa. In between the tubules, there is a testicular interstitial tissue which contains fibroblasts, collagen, blood vessels and leydig cells. Mitotic figures at different stages (prophase, metaphase, anaphase and telophase) were observed in many tubules (Figs.1&2)**.** Periodic Acid Cheif`s (PAS) reaction of the right testis showed intense reaction in well circumscribed basal lamina, strong reaction in sperms, spermatogonia and interstitial tissue which appear magenta red and mild reaction is observed in spermatocytes and spermatids (Fig. 3).

There was magenta red strong Feulgen reaction (DNA) in the nuclei of spermatogonia and sperms, moderate reaction in nuclei of spermatocytes, Leydig cells and spermatids (Fig.4). Immunohistochemical stain for caspus 3 for detection of apoptotic cells revealed negative immunoreactivity for caspus-3 in cytoplasm of spermatogenic cells, basal lamina, interstitial tissue and Leydig cells (Fig. 5).

Group II: Curcumin group:

Showed the same light microscopic appearance like control group I.

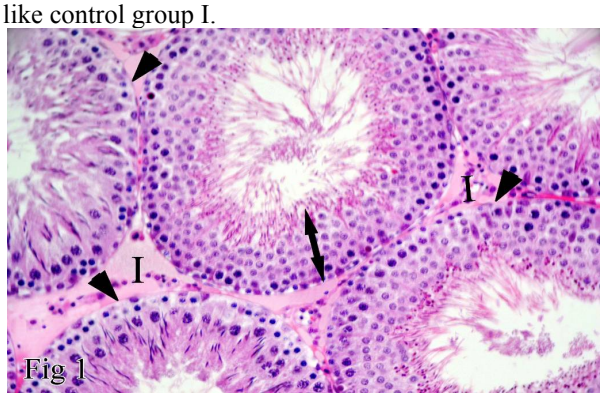

Fig.1. A photomicrograph of a control adult rat testis showing parts of five seminefrous tubules, each is bounded by a basal lamina (arrow head) and lined by normal spermatogenic cell layers (↕). Triangular interstitial tissue (I) is seen in between the tubules (H&E, X400)

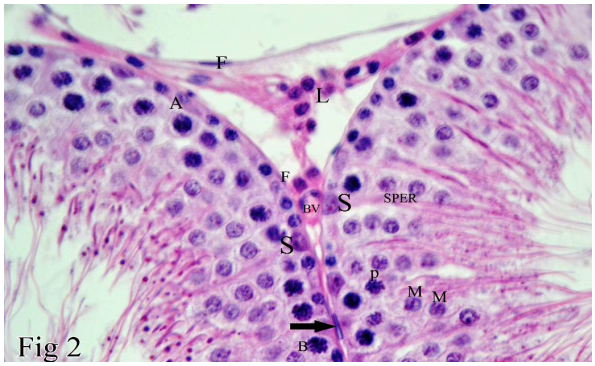

Fig.2. A photomicrograph of a control adult rat testis showing sertoli cells (S) and different spermatogenic cells; spermatogonia A (A), spermatogonia B (B), sperms (SPER). The interstitial tissue having fibroblast (F), myoid cells arrow, Leydig cells (L) and blood vessel (BV). Note: The mitotic figures as prophase (P), metaphase (M) are also seen in some spermatogenic cells. (H&E, X400)

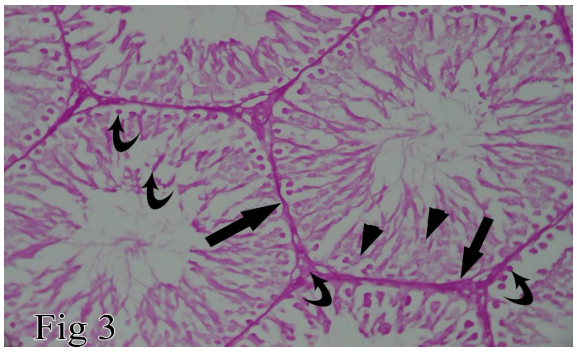

Fig. 3. A photomicrograph of a control adult rat testis showing intense PAS positive reaction in well circumscribed basal lamina (arrow), strong reaction in sperms, spermatogonia and interstitial tissue (curved arrow) and mild reaction is observed in spermatocytes and spermatids (arrow head). (PAS X400)

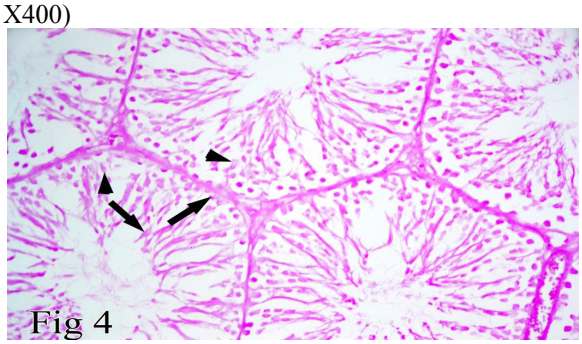

Fig.4. A photomicrograph of a control adult rat testis showing magenta red strong Feulgen reaction in nuclei of spermatogonia and sperms (arrow). Moderate reaction could be seen in nuclei of spermatocytes, leydig cells and spermatids (arrow head) (Feulgen reaction, X 400)

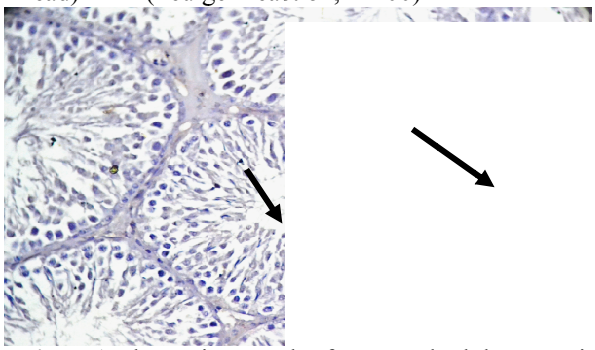

Fig.5. A photomicrograph of a control adult rat testis showing negative immunoreactivity for caspus-3 in spermatogenic cells, basal lamina, interstitial tissue and Leydig cells (arrow). (immunoreactivity to caspus-3, X 400)

Group III: Adriamycin treated group:

The seminiferous tubules of right testis sections of adriamycin treated rats showed vacuolation (V), disorganization and exfoliation of spermatogenic cells in to tubular lumen, detachment of cells from basal lamina and lined by spermatogonia. Basal lamina is thinned and absent in between some seminefrous tubules (Figs. 6 & 7). Few tubules showed accumulation of hyaline acidophilic material & streaks in tubular lumen (Fig.8). Some nuclei of spermatogenic cells appeared small and pyknotic. Interstitial tissue was widened in most areas and basal lamina appeared corrugated (Fig. 9). PAS reaction of this group showed intense reaction in the basal lamina and widened interstitial tissue, strong reaction is observed in cytoplasm of sperms, spermatogonia and moderate reaction in the other spermatogenic cells (Fig. 10). Feulgen reaction (DNA) in the nuclei of spermatogonia was mild to moderate while Weak reaction could be seen in nuclei of spermatocytes, spermatids and sperms (Fig.11). Immunohistochemical staining for detection of apoptotic cells revealed intense positive brown immunoreactivity for caspus-3 in cytoplasm of spermatogenic cells and negative immunoreactivity in basal lamina, interstitial tissue and Leydig cell (Fig. 12).

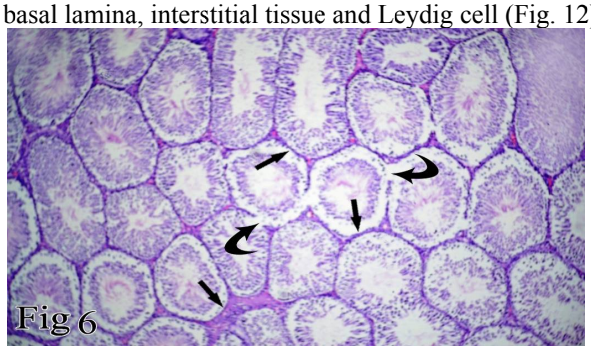

Fig.6. A photomicrograph of an adult rat testis treated with adriamycin showing many parts of semineferous tubules, with detachment of cells from basal lamina and lined by spermatogonia (arrow). Notice: Basal lamina is thinned and absent in between some seminefrous tubules (curved arrow) (H&E, X400)

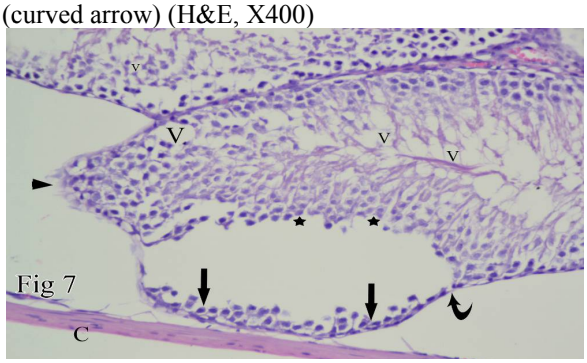

Fig.7. A photomicrograph of an adult rat testis treated with adriamycin showing semineferous tubules, with vacuolation (V), disorganization (*) and detachment of cells from basal lamina which is lined by spermatogonia (arrow).Basal lamina is thinned in some areas (curved arrow) and losted in others (arrow head). Note: Connective tissue capsule of testis (tunica albuginea) (C). (H&E, X400)

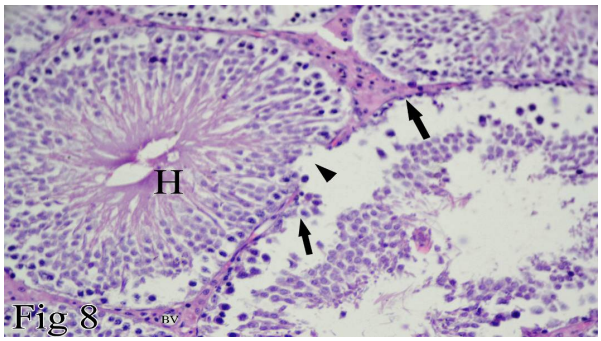

Fig.8. A photomicrograph of an adult rat testis treated with adriamycin showing semineferous tubules, with exfoliation of cells in to lumen and detachment of cells from basal lamina (arrow) which appears losted in some areas (arrow head). Notice: Red hyaline streaks in tubular lumen (H).Blood vessels (BV) in the interstitial tissue can be also seen. (H&E, X400)

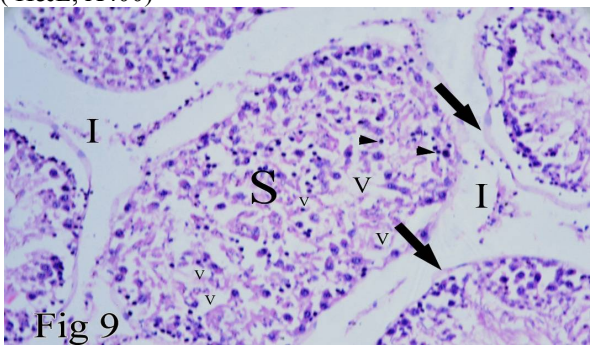

Fig.9. A photomicrograph of an adult rat testis treated with adriamycin showing semineferous tubules (S), with vacuolation (V), disorganization and exfoliation of spermatogenic cells in to lumen. Most of nuclei appeared small and pyknotic. Notice: The basal lamina appears corrugated in some areas (arrow) with wide interstitial tissue in between tubules (I). (H&E, X400)

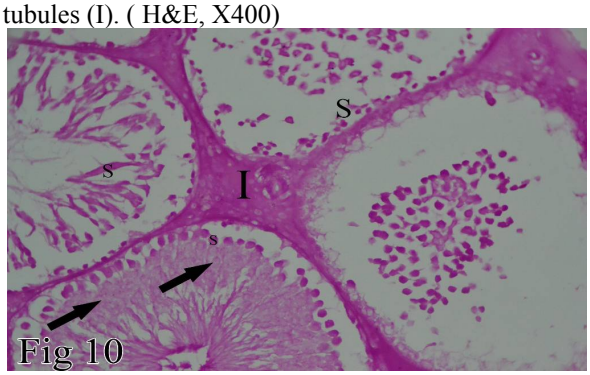

Fig.10. A photomicrograph of an adult rat testis treated with adriamycin showing very intense PAS reaction in the basal lamina and widened interstitial tissue (I), strong reaction is observed in sperms, spermatogonia (S) and moderate reaction in the other spermatogenic cells (arrow). (PAS X400)

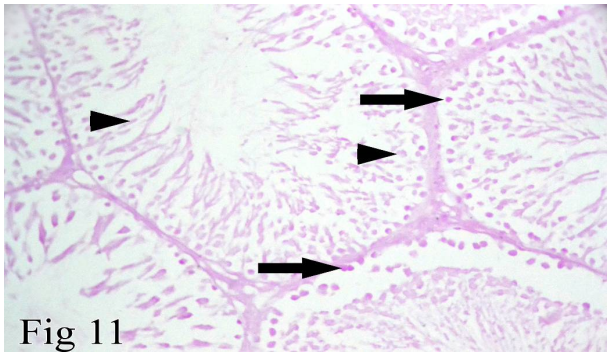

Fig.11. A photomicrograph of an adult rat testis treated with adriamycin showing mild to moderate Feulgen reaction in nuclei of spermatogonia (arrow). Weak reaction could be seen in nuclei of spermatocytes, spermatids and sperms (arrow head). (Feulgen reaction, X 400)

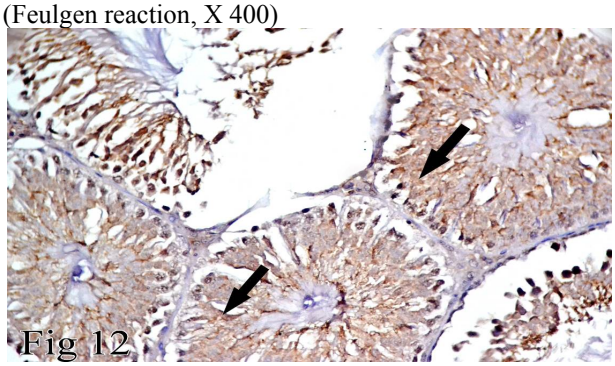

Fig.12. A photomicrograph an adult rat testis treated with adriamycin showing intense positive immunoreactivity for caspus-3 in cytoplasm of spermatogenic cells (arrow) and negative immunoreactivity in basal lamina, interstitial tissue and Leydig cell. (immunoreactivity to caspus-3, X 400)

Group IV: Protective group (Adriamycin & curcumin treated group) :

Examination of histological section of right rat testis treated with adriamycin and curcumin revealed that, most semineferous tubules looks nearly normal except for some vacuoles in interstitial tissue (V). Blood vessels (BV) were detected in interstitial tissue. in interstitial tissue. Mitotic figures could be seen in some spermatogenic cells: prophase (P), metaphase (M) and anaphase (A). The basal lamina was lifted up in some areas. (Figs. 13&14). PAS reaction of this group showed intense PAS reaction in the basal lamina and interstitial tissue, strong reaction is observed in sperms and mild to moderate reaction in spermatogenic cells. Feulgen reaction (DNA) in the nuclei of spermatogonia and sperms appeared strong and mild to moderate reaction could be seen in nuclei of spermatocytes. Immunohistochemical stain for caspus 3 for detection of apoptotic cells revealed moderate positive immunoreactivity for caspus-3 in

cytoplasm of some spermatogenic cells and strong brown immunoreactivity in cytoplasm of other spermatogenic cells (Fig.17).

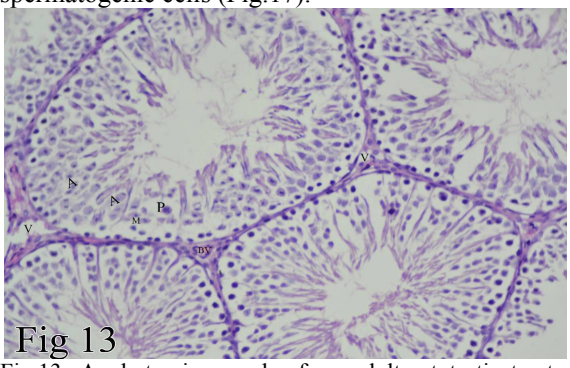

Fig.13. A photomicrograph of an adult rat testis treated with adriamycin and curcumin showing semineferous tubules looks nearly normal except for some vacuoles in interstitial tissue (V).blood vessel (BV) in interstitial tissue. Notice: Mitotic figures could be seen in some spermatogenic cells: prophase (P), metaphase (M) and anaphase (A). (H&E, X400)

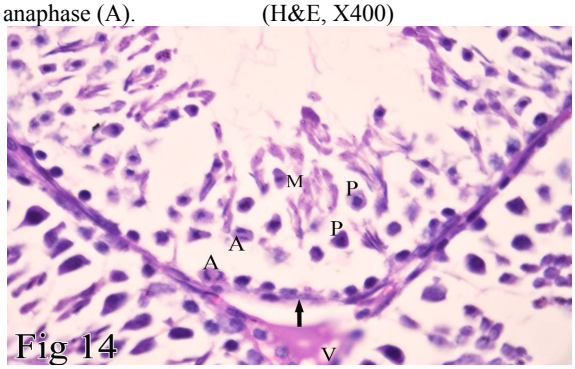

Fig.14. A photomicrograph of an adult rat testis treated with adriamycin and curcumin showing spermatogenic cells having mitotic figures: prophase (P), metaphase (M), anaphase (A) and Notice: The lifted up basal lamina (arrow) and vacuoles in the interstitial tissue (V). (H&E, X1000)

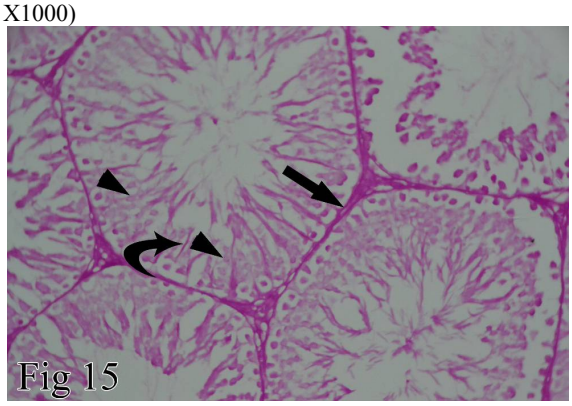

Fig.15. A photomicrograph of an adult rat testis treated with adriamycin and curcumin showing intense PAS reaction in the basal lamina and interstitial tissue arrow, strong reaction is observed in sperms (curved arrow) and mild to moderate reaction in spermatogenic cells arrow head. (PAS X400)

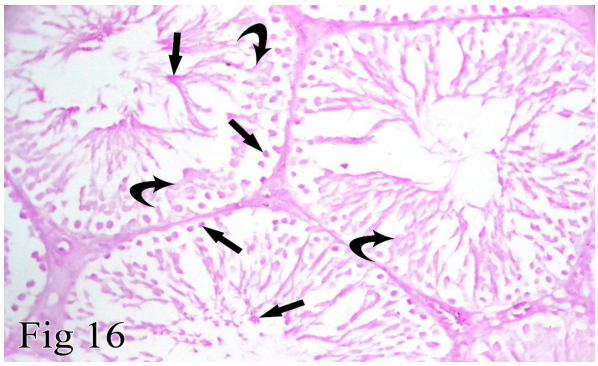

Fig.16. A photomicrograph of an adult rat testis treated with adriamycin and curcumin showing strong Feulgen reaction in nuclei of spermatogonia and sperms (arrow) and mild to moderate reaction could be seen in nuclei of spermatocytes (curved arrow) (Feulgen reaction, X 400)

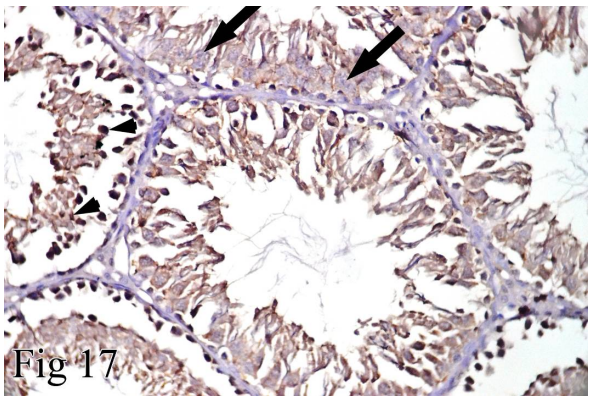

Fig.17. A photomicrograph an adult rat testis treated with adriamycin and curcumin showing moderate positive immunoreactivity for caspus-3 in cytoplasm of spermatogenic cells (arrow) and strong immunoreactivity in cytoplasm of other spermatogenic cells (arrow head). **(immunoreactivity to caspus-3, X 400)**

4. Discussion

Adriamycin is the first line anti-cancer drug in chemoresponsive tumors such as ovarian cancers, breast cancers and lymphoma (**Atessahin *et al.*, 2006**).The organ toxicity associated with adriamycin therapy limits therapeutic success of active anticancer therapy. The testis is among the non-target tissues that are vulnerable to side effects of this potent chemotherapeutic agent (**Suter *et al.*, 1997**).The occurrence of infertility following treatment with this anticancer drug is a serious concern (**Prahalathan *et al.*, 2005**).

Therefore, the aim of the present study is to assess the possible protective effect of curcumin with single dose doxorubicin induced testicular germ cell

toxic model using simple histological & histochemical methods.

In the present study, there was significant reduction in testis weight in adriamycin treated group when compared with control group. This in agreement with **Chapin *et al.*(1997)** who had the same result in his experiment, they stated that, the weight of the testis is largely dependent on the mass of the differentiated spermatogenic cells, and the reduction in the weight of the testis may be due to the reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis.

The present study revealed the normal general structure of the testis of the control rats. These findings observed by **Saalu *et al.* (2009)** who reported normal structure of testis during his experiment. This denoted that, used rats were healthy.

In the present study, histopathological examination of testis of adriamycin treated rats revealed many structural changes in seminiferous tubules in the form of disorganization and exfoliation of spermatogenic cells into tubular lumen. This can be attributed to adriamycin induced oxidative stress and production of free radicals. This agrees with findings reported by some investigators (**Endo *et al.*, 2003; Atressahin *et al.*, 2006**) who provide well documented evidences of testicular morphological impairment following adriamycin challenge in animal models. **Howell and Shaler (2001)** showed that the occurrence of male infertility following adriamycin chemotherapy is due to alterations in the sperm parameters. Spermatogenic cells constitute one of the body tissues that are susceptible to adriamycin induced oxidative stress and apoptosis by intracellular generation of free radicals and reactive oxygen species along with intercalation with DNA and subsequent inhibition of topoisomerase (**Hrdina *et al.*, 2000**). This increased oxidative stress damages the sperm membranes, proteins and DNA (**Kirsi and Timo, 2001; Kalender and Yel, 2005**).

In the current study, the seminiferous tubules showed vacuolation, some nuclei of spermatogenic cells appeared small and pyknotic. Those indicate the occurrence of degeneration and apoptosis of germ cells with appearance of vacuoles replacing cells (**Kissane, 1985**). These findings represent well known observations of toxicity of adriamycin (**Kato *et al.*, 2001**). The high dose of adriamycin induced expression change in a great number of genes related to androgens and their receptors and subsequently caused impairment of spermatogenesis (**Peters *et al.*, 2001). Shinoda *et al.* (1999)** stated that, intercalation of adriamycin in germ cell DNA during division is considered to be the principle cause of cellular death induction in the seminiferous tubules. Apoptosis and degeneration of spermatogenic cells could be confirmed by strong positive caspus 3 immunoreactivity in cytoplasm of spermatogenic cells as reported in the present study. Caspases are important components of the mammalian apoptotic cascade in mammalian cells. Caspase-dependent apoptosis is a well-characterized mechanism for removing senescent, defective, or unneeded cells (**Said *et al.*, 2004**). Caspase-3 is a prototypical enzyme that becomes activated during apoptosis in a wide variety of tissues that can degrade DNA via proteolytic activation of DNases (**Turner *et al.*, 2008**).

The Basal laminas of seminiferous tubules in adriamycin treated group was thinned and absent in between some seminefrous tubules while appear corrugated in other areas. This may be due to direct action of doxyrubcin at cell membrane level. As the drug can bind to cell membrane lipid and affect variety of membrane structure and function (**Katzung, 2007**).

In the present study, an increase in periodic acid schieff s reaction was observed in all treated groups as PAS showed intense reaction in the basal lamina and widened interstitial tissue, strong reaction is observed in cytoplasm of sperms, spermatogonia and moderate reaction in the other spermatogenic cells. This was previously reported by **Chinoy and Sharma (1998)** in their studies in rat, where glycogen content was reported to increase after treatment by adriamycin and could be explained by low level of energy production for activity of spermatogenic cells as most of them were affected. **Chinoy (1991)** showed that, the enhancement of glycogen could be explained by the reduction of phosphorylase activity, an enzyme which catalyses the conversion of glycogen into glucose -1-phosphate.

Nwankwoala *et al.* (2009) reported that, testis may belong to the family of tissues which lack or deficient in their ability to excete the drug. So, the ability of the testis to accumulate adriamycin and propability inability to excrete the drug that can facilitate toxicity.

In the current study, there was weak Feulgen reaction (DNA) in the nuclei of spermatocytes, spermatids and sperms and mild to moderate reaction in spermatogonia in adriamycin treated group denoting decreased DNA content in these cells. The decreased DNA could be explained by adriamycin effect on testicular steroidogenesis with subsequent effect on testicular DNA content causing fall in its level. **Lebrecht *et al.* (2003)** and **Serrano *et al.* (1999)** suggested that adriamycin at high dose can induce toxicity by immediate damage to mitochondrial DNA and respiratory chain and that

some mitochondrial DNA showed mutation that may persist for long time .This subsequently can generate free oxygen radicals that can induce cell injury. Adriamycin inhibit DNA synthesis via intercalation as well as generate toxic reactive oxygen species (ROS). (**Quiles *et al.*, 2002**).

In the current study, examination of the testis of the rats of the protected group which received both adriamycin with curcumin, showed that most semineferous tubules looks nearly normal except for some vacuoles in interstitial tissue. Mitotic figures could be seen in some spermatogenic cells: prophase, metaphase and anaphase. This could be attributed to curcumin protective effect as it considers being an effective antioxidant against oxidative tissue damage. It can significantly inhibit the generation of reactive oxygen species (ROS) both *in vitro* and *in vivo*. (**Duvoix *et al.*, 2005**) and so attenuate the toxic effect on rat testis, this is in line with **Salama and El-Bahr, 2007** who stated that, curcumin administration could ameliorate testicular toxicity caused by cadmium.

The antioxidant protective mechanism of curcumin on rat testis was attributed to its conjugated structure which includes two methoxylated phenols and an enol form of ß-diketone. The structure showed a typical radical trapping ability as a chain breaking antioxidant (**Masuda *et al.*, 2001**).

Curcumin had protective effect on testicular damage as it prevents peroxidative changes in the sperm and the testicular membrane, thus enhancing sperm motility and decreasing spermatozoa abnormalities. Results of the present study are in agreement with **Ishihara *et al.* (2000)** who reported the protective effect of curcumin on di-ethyl-hexylphthalate- induced testicular atrophy.

Iibey *et al.* (2009) suggested that, curcumin administration to cisplatin treated rats significantly prevented histopathologic changes induced by oxidative injury in rat testis by inhibiting mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-kB). They concluded that, curcumin has a strong potential for use as a therapeutic adjuvant in cisplatin gonadotoxicity.

Curcumin exerts a protective effect on testicular ischemia–reperfusion injury as reported *by* ***Wei et al.*** **(2009)**

Conclusion:

Toxic effect of Adriamycin should be kept in mind during chemotherapeutic treatment .Curcumin had multiple beneficial functions without any side effect. Now, it is considered the drug of the future & advised to be administered in concomitant with adriamycin treatment as it could ameliorate Adriamycin toxicity on testis.

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