Effect of Different Doses of Doxorubicin on Pituitary Gland and Some Testicular Function in Adult Male Rabbits

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Summary

The present study was designed to search out the effect of different doses of doxorubicin some functions of pituitary gland and testes in adult male rabbits. Twenty adult male rabbits were randomly divided into four equal groups and treated for 28 days as follows: first group (control) were injected with normal saline; second group (GI) were injected with doxorubicin 3 mg/ kg B.W., while animals in the third group (GII) and fourth group (GIII) injected with 3.5 and 4 mg/ kg body weight of doxorubicin respectively, all animals were injected twice a week via ear vein. At the end of experiment fasting blood (8-10 hrs) samples were collected. Blood was drawn by cardiac puncture technique and serum was collected for measuring the hormones, Follicular Stimulating Hormone (FSH), Luteinizing Hormone (LH), and Testosterone (T). In addition, sections from pituitary gland and testes were taken for histopathological studies. The results showed significant increase ($P<0.05$) in serum FSH, LH and T levels in group (GIII) as compared to GI, GI and control groups. Beside significant increases in T levels was observed in GII as compared to control group. Sever histopathological changes was observed in testes including thickness of basement membrane, protienous material in lumen of somniferous tubules and pituitary gland showed fibrosis, faculation of epithelial cell in all treated groups. In conclusion different doses of doxorubicin have detrimental effect on pituitary gland and male reproductive system of rabbits.

Keywords: Doxorubicin, Pituitary, Testicular function, Male rabbit.

Introduction

Anthracyclines is playing an important role in the various chemotherapeutic agents (1). It is derived from Streptomyces species and is tetracyclic chromophore antibiotics. Doxorubicin is one such anthracyclines which has been used effectively in treating acute leukemias and malignant lymphoma and solid tumours as well as cancers of the bladder, breast, stomach, lung, ovaries, thyroid, soft tissue sarcoma and multiple myeloma, (2 and 3). Chemotherapy for cancer is often associated with adverse effects (4). Many toxic manifestations of doxorubicin are detected such as myelosupression, thrombo- cytopenia, anaemia, stomatitis, gastrointestinal disturbances and alopecia. Many of these manifestations are reversible (1), cardiomyopathy (5) and gonadal injury by antineoplastic drugs, though commonly observed, have been relatively less investigated than their other adverse effect (6).

Doxorubicin has multiple mechanisms of action, including its interaction with the enzyme topoisomerase II, metal ion chelation and free radical generation (7 - 9). More recently doxorubicin was found to reduce the viability of cancer cells via RNA damage (10). Although doxorubicin is considered very potent and efficient chemotherapeutic drug, it also kills healthy cells, especially those under rapid and constant proliferation, such as the male germ cells. It has been shown that doxorubicin causes germ cell apoptosis (11-13).

Materials and Methods

Twenty males rabbits from local breeds used in this study which kept in special cages in animals-house of Physiology and Pharmacology Department, College of Veterinary Medicine, University of Baghdad.

Animals were divided into four equal groups (five rabbits /group) and treated for 28 days as

follows: Control group which injected with normal saline, second group (GI) were injected with doxorubicin in a dose 3 mg/kg B.W., animals in third group (GII) and fourth group (GIII) were injected with doxorubicin in a dose of 3.5 and 4 mg/kg body weight all animals were treated by injection via ear vein twice a week. At the end of experiment rabbits were scarified by withdrawal of blood from heart, by heart puncture technique, serum was collected by centrifuge (2500 rpm) for 15 mints and frozen at -20 C˚ until hormonal analysis. The testis and pituitary gland were removed and preserved in 10% neutral formalin buffer solution till the preparation of histological sections for histological studies , tissues were embedded in paraffin and several histological sections were prepared and stained with hematoxylin - Eosn (H and E) stains (14). Thickness of cell lining seminiferous tubules and diameter of seminiferous tubules where measured by oculometer (Craticules Ltd England). Follicle Stimulating Hormone concentration (mIu/ml) and LH concentration (mIu/ml) was measured by immunoradiometeric assay (IRMA) kit (Beckman coulter, immunotech), while testosterone hormone concentration (ng/ml) was measured by Radioimmunoassay (RIA) kit (Beckman coulter, immunotech).

Analysis of data was statistically performed by using analysis (ANOVA) one – way of varians and LSD was used to determine the differences between mean values. P. value (0.05) was considered significantly different (15).

Results and Discussion

Table, 1 illustrates the value of FSH concentration in the control and three treated groups of the experiment. Lack of significant (P<0.05) differences was observed in value of FSH concentration in group GI, GII and control group after 28 days of treatment when compared between each other. While there is a significant (P<0.05) increase in FSH concentration in group (GIII) comparing to control, GI and GII groups.

Table, 1: Effect of different doses of doxorubicin on FSH concentration (mIu/ml) in adult male

Groups	Parameter FSH (mIu/ml)
Control	30.34±2.34 B
Group GI	31.42±2.67 B
Group GII	42.18±3.04 b
Group GIII	69.44±6.04 a

n=5 rabbits/group; Different letters denoted significant differences between groups (P<0.05).
GI animals injected with doxorubicin 3 mg/kg B.W. twice a week.
GII animals injected with doxorubicin 3.5 mg/kg B.W. twice a week.
GIII animals injected with doxorubicin 4 mg/kg B.W. twice a week.

The concentration of LH in the control and three treated groups with doxorubicin along the experiment was clarified in table,2. The results showed a significant (P<0.05) increase in LH concentration (mIu/ml) in GIII group comparing with control group, while no significant (P<0.05) differences in LH concentration were recorded between the three treated groups (GI, GII and GIII).

Table, 2: Effect of different doses of doxorubicin on LH concentration (mIu/ml) in adult male rabbits (M±SE)

Group	Parameter LH(mIu/ml)
Control	0.10±0.02 b
Group GI	0.12±0.03 ab
Group GII	0.13±0.02 ab
Group GIII	0.17±0.02 a

n=5 Rabbits/group; Different letters denoted significant differences between groups (P<0.05).
GI animals injected with doxorubicin 3 mg/kg B.W. twice a week.
GII animals injected with doxorubicin 3.5 mg/kg B.W. twice a week.
GIII animals injected with doxorubicin 4 mg/kg B.W. twice a week.

Data pertaining to testosterone hormone concentration of control and treated groups (GI, GII, GIII) were detected in table,3.

Table, 3: Effect of different doses of doxorubicin on Testosterone concentration (ng/ml) in adult male rabbits (M±SE)

Group	parameter Testosterone (ng/ml)
control	7.37±0.77 b
Group GI	5.46±1.40 b
Group GII	20.26±0.64 a
Group GIII	20.35±1.10 a

n=5 rabbits/group; Different letters denoted significant differences between groups (P<0.05).
GI animals injected with doxorubicin 3 mg/kg B.W. twice a week.
GII animals injected with doxorubicin 3.5 mg/kg B.W. twice a week.
GIII animals injected with doxorubicin 4 mg/kg B.W. twice a week.

The results revealed that there is no significant (P<0.05) differences in the mean value of testosterone concentration between control and GI groups. A significant (P<0.05) increase in testosterone concentration were detected at the end of experiment in GII and GIII groups comparing to control and GI groups.

The present study showed a rise in the concentration of testosterone in groups GII and GII following with treatment of Doxorubicin obtained following acute injury, several mechanisms may account for such a change. Johnson and Ewing (16) have demonstrated that FSH augments testosterone secretion stimulated by LH in rabbit testes perfused *in vitro* with an artificial medium. There was a rise in FSH that could have brought about such an effect. In addition, De-Kretser *et al.*, (17) have demonstrated in rats that *in vitro* hyper responsiveness of testosterone secretion to human choriomc gonadotrophin could be induced by experimental cryptorchidism, and have suggested that local factors resulting from the disruption of spermatogenesis are responsible for such hyper responsiveness. However, the elevation in LH levels observed would in itself be sufficient to cause the associated rise in testosterone values. It is known that in the male, feedback regulation of LH is controlled by testosterone, the feedback being negative (18).

Chapman *et al.* (19) noted two Hodgkin's patients with normal LH and low testosterone values before treatment; after chemotherapy, rising LH levels were accompanied by a persistent rise in testosterone into the normal range, again demonstrating, as in the present paper, an abnormal relationship between LH and testosterone. It might be that another mechanism controls of LH secretion and that it is disrupted during gonadal injury.

The mechanism would be independent of testosterone analogous to the control of FSH by inhibin. In fact, inhibin could be involved in this mechanism, since it has been suggested that this hormone may be involved in the regulation of not only FSH, but also LH (20 and 21). Dosage to the seminiferous tubules induced by chemotherapy could result in decreased inhibin production, which in turn would cause an increase not only in FSH but also in LH. Depending on the results (table,4).

Table, 4: Effect of different doses of doxorubcin on diameter of seminiferous tubules

Group	parameter diameter of seminiferous tubules (mm)
Control	0.233±0.007 a
Group GI	0.204±0.008 b
Group GII	0.169±0.010 c
Group GIII	0.166±0.004 c

n=5 rabbits/group; Different letters denoted significant differences between groups (P<0.05).
GI animals injected with doxorubicin 3 mg/kg B.W. twice a week.
GII animals injected with doxorubicin 3.5 mg/kg B.W. twice a week.
GIII animals injected with doxorubicin 4 mg/kg B.W. twice a week.

There is a significant (P<0.05) decrease in mean values of diameter of seminiferous tubules (millimeter) in GI, GII and GIII treated groups comparing with control group. Insignificant differences were observed in the mean value of the parameters in both treated groups GII and GIII when compared between each other.

There were no significant differences in the mean value of thickness of cell lining seminiferous tubules (millimeter) thickness between GI and control groups along the experiment period (table,5). A significant ($P<0.05$) decrease in this parameter was detected in GII and GIII after 28 days of treatment with doxorubicin comparing to control group.

The histopathological examination of pituitary gland of treated animals treated with 4mg/kg B.W of doxorubicin showed lesion characterized by sever thickness of capsular layer due to proliferation of fibrous connective tissue which leads to atrophy of gland parenchyma which showed faculation of the cells (figure,1), While the lesion was between sever faculation of cytoplasm of the cells and edema in animals which treated with 3.5mg/kg doxorubicin (figure,2) to moderate faculation in epithelial cells with edema in animals which treated with 3 mg/kg doxorubicin (figure,3).

Table, 5: Effect of different doses of doxorubicin on Thickness of cell lining Seminiferous Tubules (millimeter) in adult male rabbits

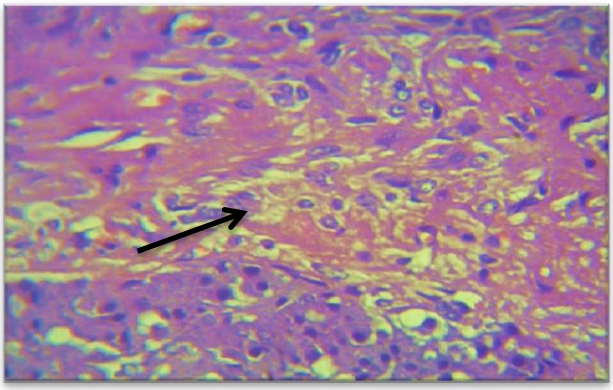
Group	parameter Thickness of cell lining seminiferous tubules (mm)
Control	0.113±0.02 a
Group GI	0.076±0.008 ab
Group GII	0.064±0.004 bc
Group GIII	0.029±0.004 c

n=5 rabbits/group; Different letters denoted significant differences between groups (P<0.05).
GI animals injected with doxorubicin 3 mg/kg B.W. twice a week.
GII animals injected with doxorubicin 3.5 mg/kg B.W. twice a week.
GIII animals injected with doxorubicin 4 mg/kg B.W. twice a week.

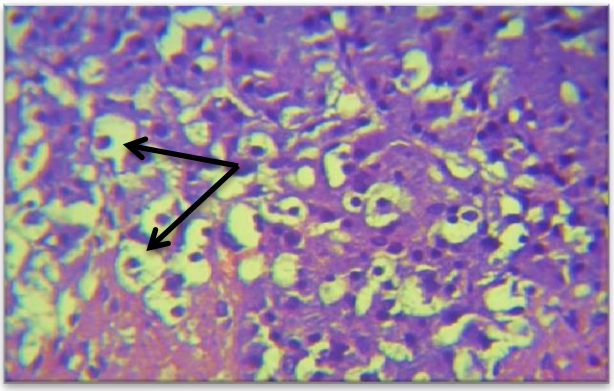
The histopathological examination of testes animals treated with 4mg/kg B.W of doxorubicin showed sever destruction of the spermatogonia of seminiferous tubules which characterized by thickness of basement membrane and the seminiferous tubules was lining only with basement membrane with faculation and necrosis of the Sertoli cells and spermatogonia. (figure,4) The microscopic examination also showed thickness of basement membrane of the somniferous tubules which a show atrophy and necrosis of spermatogonial layer animals treated with 3.5mg/kg doxorubicin (figure,5) while no clear pathological lesion seen in somniferous tubules in animals treated with 3mg/kg doxorubicin except protienous material in lumen of somniferous tubules without sperms (figure,6).

Present result is in agreement with (22, 23, 24, 25 and 26). In previous studies a considerable reduction of the seminiferous epithelium height and volume density was observed in pubertal and adult rats that were treated with doxorubicin. Patil and Balaraman (26) have reported vacuolization and fibrinoid debris in the seminiferous tubule when male rats were treated with 15 mg/kg of Doxorubicin. Besides, severe degenerative changes in germinative cells, atrophy in the diameter size of seminiferous tubules and germinative cell thickness was observed by (27)

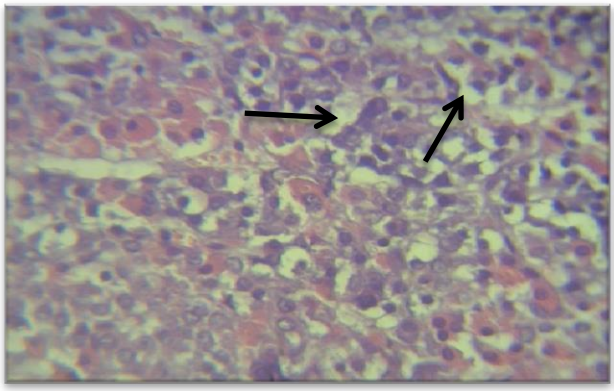
Oxidative stress and free radical production by doxorubicin may be lead to these histopathological changes (28). Mishra and Bhiwgade (29) reported that higher levels of H_2O_2 can be converted, in part, by Fenton reaction to OH· which may lead to lipid peroxidation and DNA cross-linking. These results of previous investigators could explain the present observations related to doxorubicin-induced oxidative stress via increasing lipid peroxidation through the impairment of SOD/ GPX and/or catalase ratio. It also gave reasons for damages and histopathological complications recorded in present study.



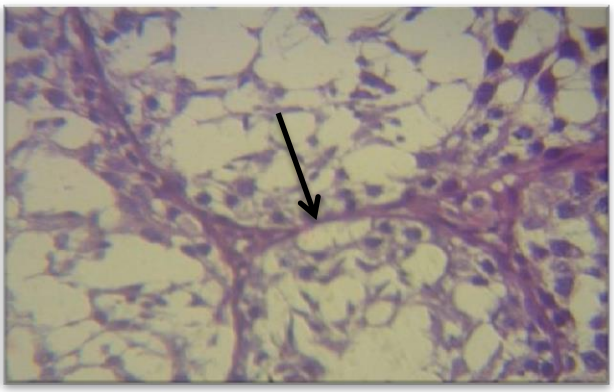
Figure, 1: Histological section in the pituitary gland of rabbits (GIII) at 28 days post treated with 4mg/kg B.W of doxorubicin shows sever fibrosis with atrophy and faculation of pituitary cells (H and E 40X).



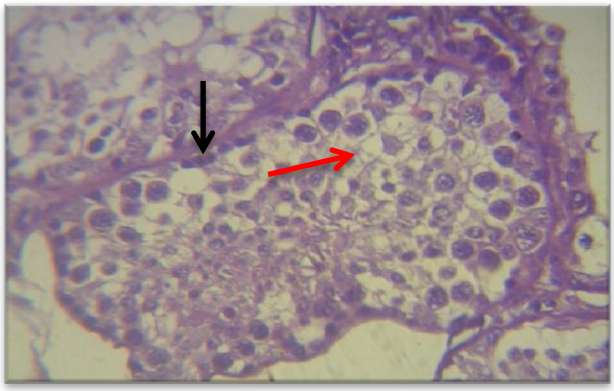
Figure, 2: Pituitary gland of rabbits (GII) at 28 days post treated with 3.5mg/kg B.W of doxorubicin shows sever faculation of epithelial cells with edema (H and E 40X).



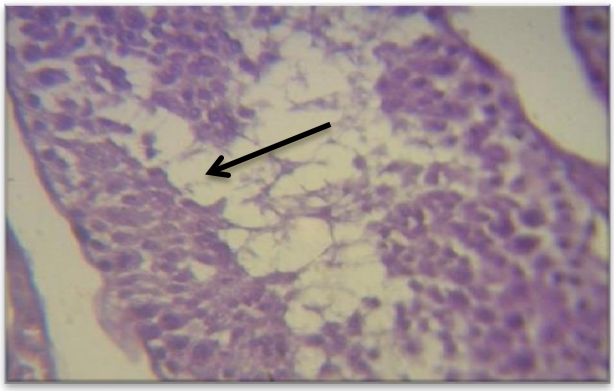
Figure, 3: Pituitary gland of rabbits (GI) at 28 days post treated with 3mg/kg B.W of doxorubicin shows moderate faculation with edema in the cortex of pituitary gland (H and E 40X).



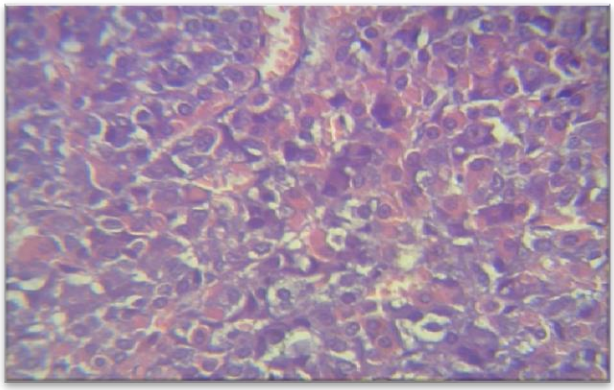
Figure, 4: Testes of rabbits (GIII) at 28 days post treated with 4mg/kg B.W of doxorubicin shows thickness of basement membrane faculation spermatogonia (H and E 40X).



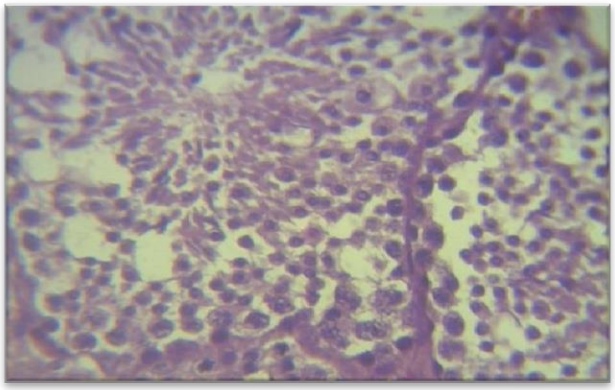
Figure, 5: Testes of rabbits (GII) at 28 days post treated with 3.5mg/kg B.W of doxorubicin shows thickness of basement membrane due to fibrosis, → necrosis and atrophy of spermatogonia → (H and E 40X).



Figure, 6: Testes of rabbits (GI) at 28 days post treated with 3mg/kg B.W of doxorubicin shows protienous material in lumen of somniferous tubules without sperms (H and E 40X).



Figure, 7: Normal structure of pitiutary gland in control group (H and E 40X)



Figure, 8: Normal structure of testes in control group with sperms in lumen (H and E 40X).

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تأثير جرع مختلفة من عقار الدوكسوروبسين على بعض وظائف النخامية والخصى في ذكور الارانب البالغة

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الخلاصة

صممت هذه التجربة لدراسة تاثير تراكيز مختلفة من عقار الدوكسوروبسين على بعض وظائف الغدة النخامية والخصى في ذكور الارانب البالغة. استعمل في هذه الدراسة عشرون من ذكور الارانب المحلية البالغة ، قسمت عشوائيا الى اربعة مجاميع متساوية وعوملت كما يلي: المجموعة الاولى ، مجموعة السيطرة حقنت بالمحلول الفسلجي الطبيعي, المجموعة الثانية (G1) حقنت بالدوكسوروبسين بتركيز (3ملغرام/كغم وزن الجسم) اما المجموعة الثالثة (GII) فقد حقنت بالدوكسوروبسين بتركيز (3.5 ملغرام / كغم وزن الجسم) و حقنت المجموعة الرابعة(GIII) بالدوكسوروبسين بتركيز (4 ملغرام / كغم وزن الجسم)جميع المعاملات كانت عن طريق الحقن في الوريد الأذني بواقع مرتين في الاسبوع ولمدة ثمانية عشرون يوم. تم سحب عينات الدم عن طريق الوخز القلبي بعد تجويع الحيوانات لمدة 8-10 ساعات وعزل مصل الدم وحفظ في المجمدة لغرض قياس تركيزالهرمون اللوتيني (LH) والهرمون المحفز للجريب (FSH) وكذلك هرمون الشحمون الخصوي(T) ، كما اخذت عينات من الغدة النخامية والخصية لغرض اجراء الفحص النسيجي. اظهرت النتائج زيادة معنوية (P<0.05) في تراكيزالهرمونات LH و FSH و T في المجموعة الرابعة (GIII) مقارنة مع مجموعة السيطرة، كما لوحظ وجود زيادة معنوية (P<0.05)في تركيز هرموني فضلا عن الى تغيرات نسجية واضحة تمثلت بانخفاض اقطار النبيبات المنوية وسمك طبقة الخلايا كذلك خزب وتنخر الخلايا في حيوانات التجربة الثانية والثالثة والرابعة,نستنتج من ذلك ان لعقار الدوكسوروبسين تاثيرا واضحا على محور النخامية-الخصى في الارانب البالغة.

الكلمات المفتاحية: الغدة النخامية ،وضائف الخصى ، دوكسوروبسين، ذكر الارنب.