

# Effect of Doxorubicin on the Histological Structure of the Liver in Male Albino Rats

Mohammed T. Taher<sup>\*1</sup>, Maha A. Al-Sammak<sup>1</sup>, Maysoon M. AL-Qzazz<sup>1</sup>

## Abstract

**Objective:** This study is concerned with the effect of doxorubicin on the histological structure of the rats livers.

**Animals studies and drug treatment:** for this experiments 24 albino male rats ( ranging from 200-220g ) body weight and are three months age were taken. For this purpose three groups each consists of 8 male rats were examined. The animals were allowed to acclimatize to laboratory conditions one week prior to the experiments. Group I was left as a control, while group II received intraperitoneally a low dose of 0.2 mg /Kg doxorubicin, group III received the therapeutic dose of doxorubicin of 1mg /Kg intraperitoneally.

Intraperitoneal injection of doxorubicin was given in the lower lateral part of the abdomen. The animals were scarified at the end of the experiment and the livers were collected from all groups to be prepared for light microscopic examination.

**Results:** Light microscopic observations revealed that higher doses of doxorubicin caused massive hepatotoxicity including dissolution of the hepatic cords, focal inflammation, apoptosis and necrosis of the hepatic tissues with fibrosis around the portal area. Lower doses exhibited abnormal changes including vacoulation of the hepatocytes with widening of the sinusoidal capillaries in addition to congestion and vasodilatation of the central veins.

**Conclusions:** This study revealed that doxorubicin causes marked changes in rats liver; which occur even in low doses; such results can guide the design of appropriate treatment regimens to reduce the hepatotoxic effects of this anticancer drug such as the concomitant use of antioxidant drugs.

**Keywords:** doxorubicin, rat liver, cytotoxic drugs.

(J Med J 2013; Vol. 47 (3):220- 226)

Received

Feb. 8, 2012

Accepted

March 21, 2013

## Introduction

Quinine- containing anthracycline antibiotic doxorubicin (DXR) has been used for the treatment of a wide variety of cancers, despite of its high antitumor efficacy DXR use in chemotherapy has been largely limited due to

its cardiac, renal and hepatic toxicity<sup>(1,2)</sup>.

Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor, however the cytotoxic drugs do not distinguish between a cancer cell and a normal cells and not only the fast growing

1. Department of Anatomy and Histology, College of Medicine, University of Mosul, Iraq.

\* Correspondence should be addressed to:

Mohammad T. Taher M.B.Ch.B.M Sc.PhD

\* m-Iden@yahoo.com

cancer cells but also other normal fast growing cells in the body including hair and blood cells will be affected<sup>(3)</sup>. More than half of all patients diagnosed with cancer received chemotherapy regime, that usually include drugs to treat cancer as well as drugs to help support the completion of the cancer treatment at the full dose on schedule<sup>(4)</sup>. Doxorubicin drug exhibits severe toxicity and undesirable side effects, the success of cancer chemotherapy is critically dependent on the ability of the chemotherapeutic agents to induce effective apoptosis in the target cancer cells<sup>(5,6)</sup>.

The basic structural unit of the liver is the hepatocytes, those cells are grouped in interconnected plates. Each classical liver lobule is formed of a polygonal mass of tissue in which the hepatocytes are radially disposed. Between those cells a sinusoidal liver capillaries and bile canaliculi are present. At the center of each hepatic lobule there is a central vein. Portal areas are present at the periphery of each lobule and are occupied by the portal triads which consist of a branch of the portal vein, a branch of the hepatic artery and a branch of the bile duct system<sup>(7,8)</sup>.

Doxorubicin has been used for more than thirty years for the treatment of various malignancies including breast tumors, bile duct, endometrial tissues, esophagus, liver as well as bone tumors<sup>(9)</sup>.

#### ***Animals studies and drug treatment***

For this experiments 24 albino male rat (ranging from 200-220g) body weight and three months in age were taken from animal house of Experimental Research Unit, College of Medicine, University of Mosul, Iraq. The animals were allowed to acclimatize to laboratory conditions one week prior to the

experiments and were caged in a quite temperature controlled room ( $23 \pm 4^\circ\text{C}$ ) they were allowed to drink tap water freely and fed daily by pellet foods including 21% pure protein under optimum laboratory conditions. The animals were divided into three experimental groups (n=8 each) and each group was given the following treatment:

Group I: control rats were left with intraperitoneal administration of normal saline weekly for one month.

Group II: rats have been given low dose of 0.2mg /kg body weight doxorubicin intraperitoneally weekly for one month.

Group III: rats have been given therapeutic dose of doxorubicin 1mg/kg body weight weekly for one month.

Intraperitoneal injection was given in the lower lateral part of the abdomen. The animals were sacrificed at the end of the experiment which lasted for one month under light ether anesthesia.

#### ***Rat dissection***

Incision in the rat skin was made at the mid point of the neck and continues downward toward the tail, using a central line to cut a similar pattern then the cutting passes through the abdominal wall muscle to the peritoneum the liver was exposed it is a large structure located beneath the diaphragm at the right hypochondrium.

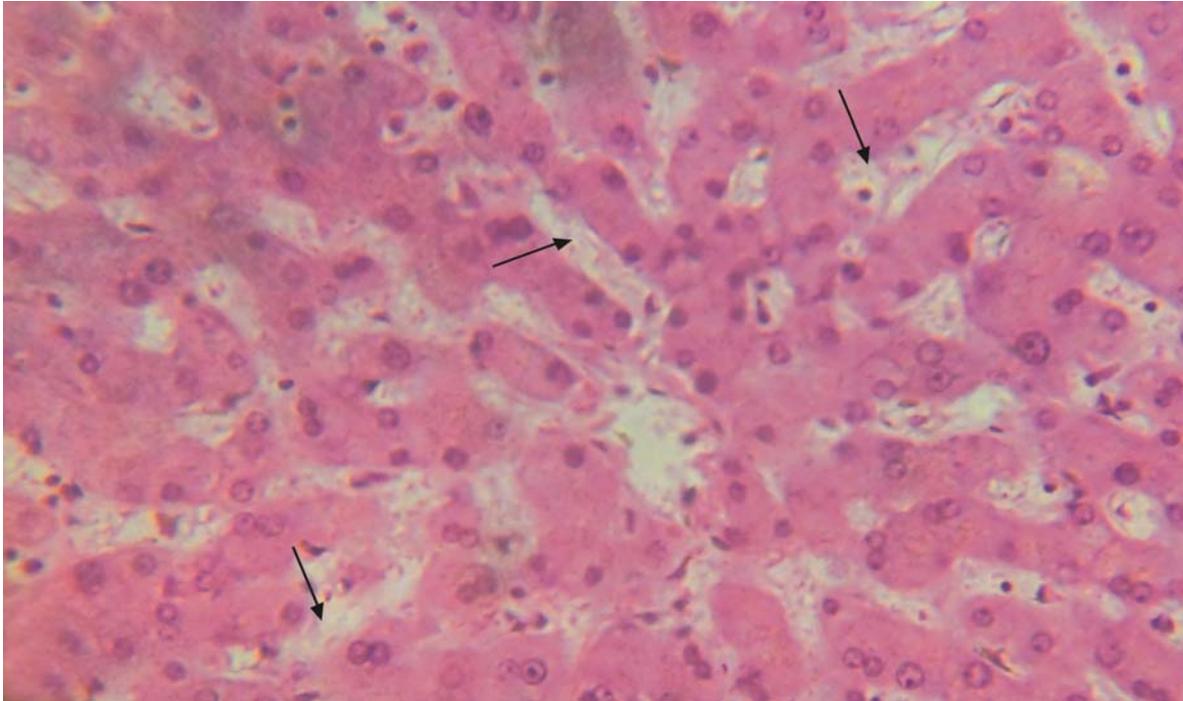
The livers were collected from all groups, fixed in 10% neutral buffered formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and mounted in paraffin wax with melting point  $58-62^\circ\text{C}$ . five microns sections were obtained from these blocks which were stained with Harris Hematoxylin and Eosin and Van Gieson stains to demonstrate fibrosis.

## Results

### Light microscopic

Observation of the hepatic tissues of the control group showed normal large polygonal

hepatocytes with rounded prominent nuclei and eosinophilic cytoplasm and few spaced hepatic sinusoids arranged in between the hepatic cords around the central veins fig.(1).



**Figure 1: Normal sinusoidal spaces between the hepatocytes (arrows) (H&E 100x)**

In contrast, in the group receiving the low dose (0.2 mg/kg) of doxorubicin the most pronounced histological abnormalities observed were vacuolation of the hepatocytes and apoptosis mainly around the central vein fig.(2), widening of the sinusoidal spaces between the plates of hepatocytes in addition to mild congestion and vasodilatation of central veins fig.(3).

In addition to the previous changes the group which received therapeutic dose of doxorubicin (1mg/kg) showed dissolution of the hepatic cords around the central vein fig.(4) with chronic inflammatory cells infiltration mainly lymphocytes and plasma cells between the hepatocytes fig.(5) with marked necrosis of the

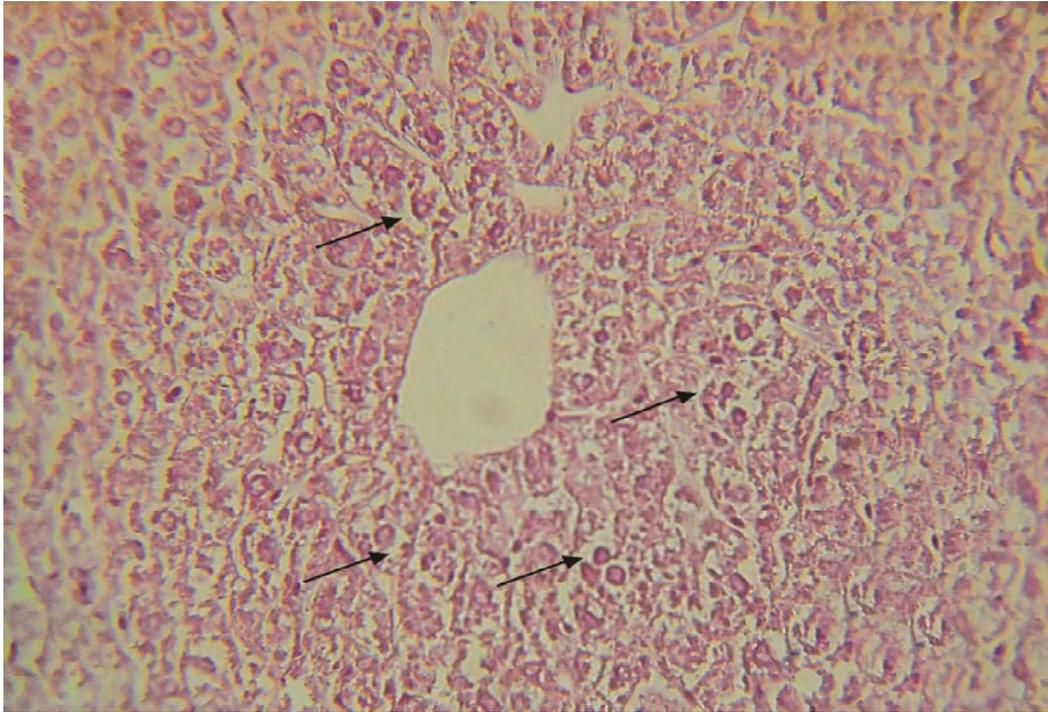
hepatocytes around the central vein which extend to involve the peripheral cells fig.(6) and extensive hepatic fibrosis seen clearly in the portal area fig.(7).

### Discussion

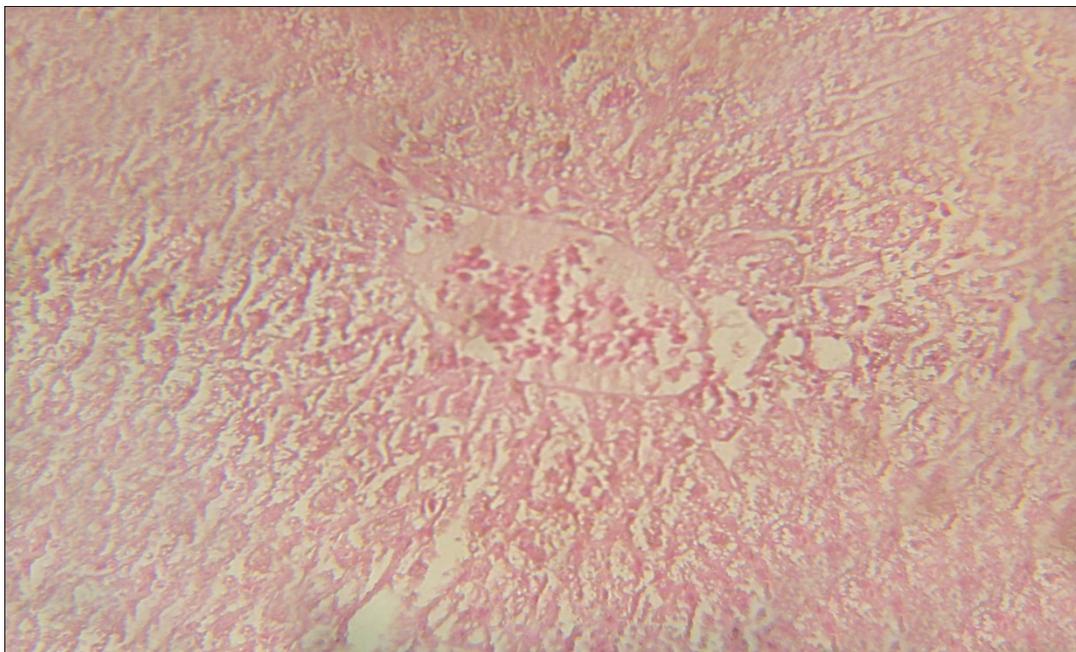
Doxorubicin is firmly established as a major therapeutic agent in the treatment of a wide variety of tumors. Although the precise mode of antitumor action of this drug is not well established, it is thought to involve the interference with the synthesis of macromolecules, covalent DNA binding and DNA cross-linking, inhibition of topoisomerase II, arrest of tumor cell cycle progression in G2 phase, induction of

apoptosis and generation of reactive oxygen radicals<sup>(11)</sup>. Two of these phenomena require enzymatic activation, including

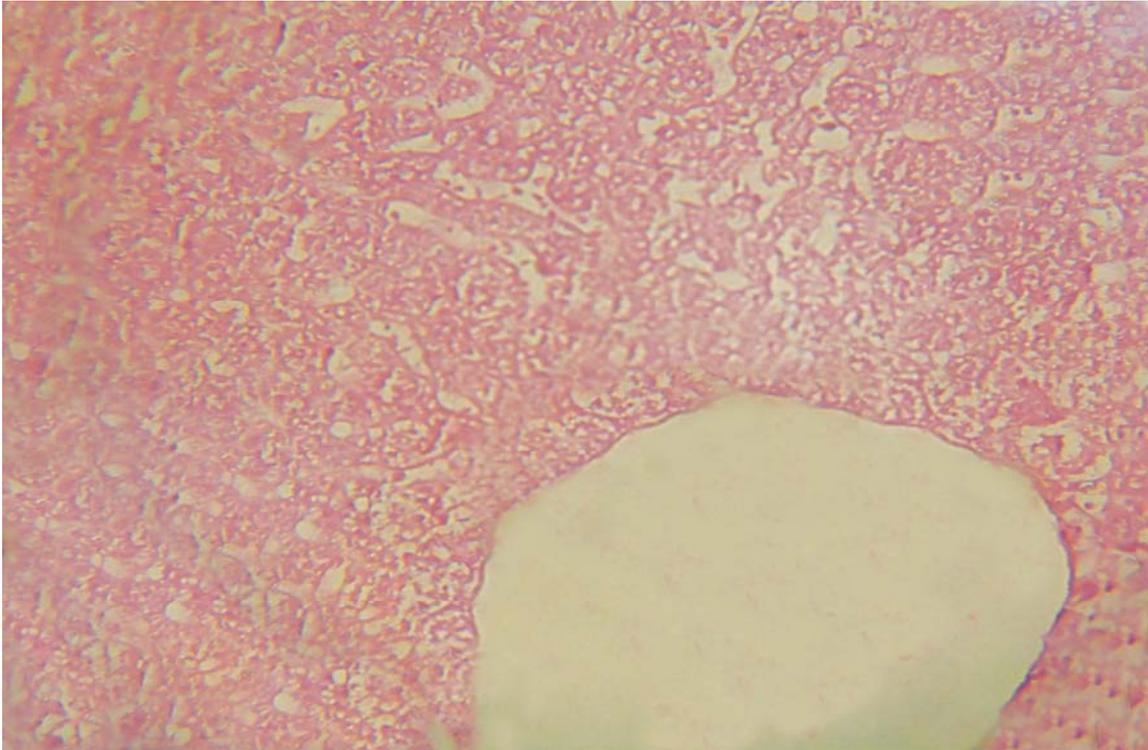
covalent modification of macromolecules and redox cycling with reactive oxygen species and both of these effects can cause cytotoxicity<sup>(12)</sup>.



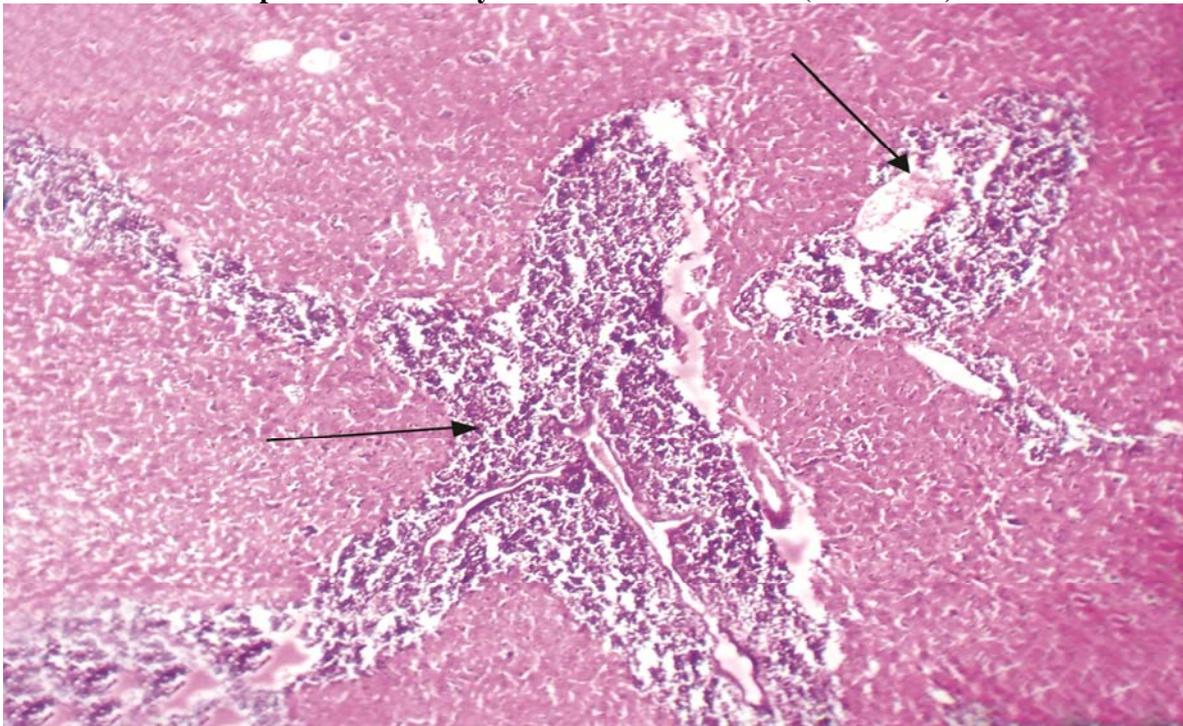
**Figure 2: Rat liver from the low dose group with vacuolation of hepatocytes (arrows) and central apoptosis (H&E 400x)**



**Figure 3: Rat liver from the low dose group with widening of sinusoidal spaces, congestion and vasodilatation of the central vein (H&E 400x)**



**Figure 4: Rat liver from the therapeutic- dose group with dissolution of hepatic cords mainly around the central vein (H&E 400x)**



**Figure 5: Rat liver from the therapeutic-dose group with chronic inflammatory cells infiltration (lymphocytes and plasma cells) in the sinusoids and around the central vein (arrows) (H&E 100x)**

In this study marked histological changes were observed in both groups of rats. Vacuolation of the hepatocytes was observed in the group which received the low dose of doxorubicin; it is well known that the hepatocytes are very active metabolic cells and when such cytotoxic drug is used, it affects the cell cycle and kills the cells primarily by forming DNA adducts causing G2 arrest which leads to disturbances in its metabolic activities which in turn leads to shape distortion, possibly due to edema and accumulation of fluid within the cells. Similar changes were also observed by other workers and some of whom considered the disturbances in the function and shape of the hepatocytes due to the DNA damage in the liver cells provoked by doxorubicin<sup>(13)</sup>.

Many hepatocytes showed karyomegaly and pyknotic nuclei indicating apoptosis, the liver is known to accumulate significant amount of this drug. Cell death can result either from naturally occurring apoptosis (physiological apoptosis) or from irreparable cell injury (pathological apoptosis). Apoptosis is a common feature of hepatotoxicity induced by many anti-carcinogenic drugs. It may precede necrosis or it may occur concurrently with necrosis as described by Faber (1994)<sup>(14)</sup>. So in addition to the potentiation of tumor chemotherapy doxorubicin likely to enhance cytotoxicity against normal tissues<sup>(11)</sup>. A variety of agents including anti-oxidants have been shown to attenuate the hepatotoxicity of these compounds<sup>(15)</sup>.

Microscopic examination also revealed intense infiltration of hepatic tissues with mixed inflammatory cells mainly lymphocytes and plasma cells with liver macrophages. These cells leave the blood and enter the tissues as a part of immunological response of the body to the inflammation following congestion and vasodilatation of the central vein. Doxorubicin

has been shown to induce accumulation of inflammatory cells associated with increased activities of tissue amino-transferases, lactate dehydrogenase and alkaline phosphatase enzymes indicating hepatic damage<sup>(16,17)</sup>. As a result of dense collection of plasma and lymphocytes and tissues destruction induced by these cells, fibrocytes will cause accumulation of collagen fibers mainly in the portal area, manifested as an early sign of fibrosis. These changes were also mentioned by other authors<sup>(13)</sup>.

This study revealed marked toxic effect of doxorubicin on the rat's liver at the cellular levels. We suggested a follow up study using antioxidant agents to prove that their use decreases the toxicity of the anticancer drugs.

## References

1. P.K Single, C.M.R. Deally, and L.E Weinberg. Subcellular effect of doxorubicin. *Journal of Molecular and Cellular Cardiology*, 1999; 19, (8): 817-828.
2. E. Fadilliogiu, H. Erdogan, S. Sogut and I. Kuku. Protective effect of erdosteine doxorubicin induce cardiopathy in rat. *Journal of Applied Toxicology*, 2003; 23 (1): 74.
3. Bonadonag G, Valagussa P, Moliterna A, Zambetti M, Brambilla C. Adjuvant cyclophosphamide methotrexate and fluorouracil in node-positive breast cancer. *Engl J Med*. 1995; 332: 901-906.
4. De Graaf H., Willemse PH. Intensity of standard adjuvant CSF with granulocyte colony stimulating factor for premenopausal patients with node-positive breast cancer. *Oncology*, 1996; 53: 289-294.
5. Schmitt C. A., Lowe S. W. Apoptosis and therapy. *J. Pathol*. 1999; 187:127-137.
6. Kastan M.B. Molecular determinants of sensitivity to anti-tumor agents. *Biochim. Biophys. Acta*. 1999; 1424: 37-42.
7. Ganquerra LC, Carneiro J, Long JA. *Basic histology 10th edition*. Lange Medical Publications, California, USA., 2003; 309-325.
8. EL-Rakhawy MT. *General embryology*. 1st ed., WHO, Regional Office Eastern Medetran., Egypt, 2000; 108-112.

9. Sule Ayla, Ismail Seckin and Gulperi Oktem. Doxorubicin induced nephrotoxicity: protective effect of nicotinamide. International Journal of Cell Biology, 2011; 2011: 1100-1155.
10. MacManus JFA. and Mowry RW. Staining Histological and Histochemical. 1st ed. Harper and Row, New York, USA, 1964; 74, 126.
11. Lubgan D. Marczak A, Distel L. Pharmacological mechanisms of doxorubicin activity. Current State of Knowledge, 2006; 63: 782-800.
12. Lai PS, Pai CL, Yen WN. Doxorubicin delivered by polyamidoamine dendrimer conjugation and photochemical internalization for cancer therapy. J. Control Release, 2007; 122: 39-46.
13. Hassan I El-Sayyad, Mohamed F Iasmil, F M Shalaby, R F Abou-El Magd, Rajiv L Gaur, Augusta Fernando, Madhwa HG Raj and Allal Ouhtit. Histopathological effects of cisplatin, doxorubicin and 5- flurouracil (5-Fu) on the liver of male albino rats. Journal List. Int. Biol. Sci., 2009; (5): 1-9.
14. Faber E. Programmed cell death: necrosis versus apoptosis. Mod. Pathol., 1994; 7: 605-609.
15. Ray S., Roy K. and Sengupta C. Invitro evaluation of protective effects of ascorbic acid and water extract of spirulina plantesis on 5-Fluorouracil induced lipid peroxidation. Acta Pol Pharm. 2007; 64: 335-344.
16. Saad Sy, Najja TA., Alrikabi AC. The preventive role of deferoxamine against acute doxorubicin -induced cardiac, renal and hepatic toxicity in rats. Pharmacol Res., 2001; 43: 211-218.

## تأثير عقار الدوكسوروبيسين على التركيب النسيجي لكبد الجرذان

محمد الطاهر\*، مها السماك، ميسون القزاز

كلية الطب، جامعة الموصل، العراق

### الملخص

**الهدف:** يهدف هذا البحث إلى دراسة تأثير عقار الدوكسوروبيسين على التركيب النسيجي لكبد الجرذان. لهذا الغرض؛ تم أخذ 3 مجاميع من الجرذان، كل مجموعة تتكون من 8 جرذان: المجموعة الأولى تركت كمجموعة ضابطة، والمجموعة الثانية تم حقنها داخل غشاء البريتون بجرعة صغيرة 0,2 ملغم لكل كيلوغرام من وزن الجسم، أما المجموعة الثالثة؛ فقد تم حقنها بالجرعة الدوائية، وهي 1 ملغم لكل كيلوغرام من وزن الجسم. أظهرت هذه الدراسة سمية هذا العقار على كبد الجرذان باستعمال المجهر الضوئي، ولوحظ أن استعمال جرعة كبيرة من الدوكسوروبيسين سبب تأثيراً عالي السمية على خلايا الكبد، يتضمن فقدان الشكل المنتظم لصفائح الخلايا الكبدية مع التهاب موضعي، وتخر في أنسجة الكبد. أما الجرعة القليلة؛ فقد أدت إلى تحول خلايا الكبد إلى خلايا فقاعية مع توسع الأوعية الشعرية الكبدية ووجود احتقان ونزف في الوريد المركزي لأنسجة الكبد. وأظهرت هذه النتائج أن عقار الدوكسوروبيسين سبب تغيرات كبيرة في كبد الجرذان حتى في الجرعة القليلة. وهذه النتائج يمكن أن تساعدنا في اختيار الطرق المناسبة للتقليل من سمية هذا العقار، كإضافة عقارات مضادة للأكسدة.

**الكلمات الدالة:** الدوكسوروبيسين، كبد الجرذان، عقار سام للخلايا.