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BIOCHEMICAL AND HEMATOLOGICAL ALTERATIONS ASSOCIATED WITH DOXORUBICIN INDUCED TOXICITY IN RAT

Yakout, A. El-senosi[a]; Samy Ali. Hussein[a]; Sayed Bakry[b] and Mariam Thabit[a]*.

[a]*Biochemistry department, Faculty of Veterinary Medicine, Banha University - Egypt*
[b]*Center for Genetic Engineering – Al-Azhar University, Cairo - Egypt*

*Corresponding Author: mariamthabit@hotmail.com
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ABSTRACT

Doxorubicin is an anthracycline drug first extracted from *Streptomyces peucetius var. caesius* in the 1970's. It used routinely as highly efficacious drug in many kinds of cancers. But its clinical usefulness is still restricted due to its specific toxicities. This study was designed to investigate doxorubicin-induced toxicity in albino rat model. Thirty male young adult Sprague Dawley rats were divided into Three groups. The first served as control group while second and third group treated with 2.5 and 5mg / kg /week. Two hours post DOX administration rats from all the groups were killed, blood samples were drawn for hematological and biochemical changes. Data were analyzed using (SPSS, verson 22). Hematological parameters obtained from rats treated with Dox revealed that Dox doses induced decrease in RBCs Count, Hemoglobin (Hb) concentration and HCT % when compared with the corresponding values of control rats. Otherwise, WBC.s differential count including (lymphocyte, monocyte, neutrophil, monocyte as well as neutrophil) and platelets count showed a significant decrease (P<0.05) when compared with the values of control rats. DOX doses for four weeks induced hepatic damage as reflected by significantly ($p < 0.05$) elevated serum ALT, TSB, and ALP enzymes activities when compared to control group. Albumin concentrations recorded a significant decrease. While serum urea concentration and creatinine levels were elevated (P<0.001) as compared with the values of control rats. The present study shed more of the light on the effect of Doxorubicin-induced toxicity in albino rat model. Showing several biochemical and hematological abnormal effects.

Keywords: Doxorubicin; Hematology; liver functions; kidney functions.

1. Introduction

Doxorubicin is an anthracycline drug that was first isolated in the 1970s from *Streptomyces peucetius var. caesius*. Doxorubicin is a highly effective medication that is commonly used to treat a variety of cancers. However, due to its unique toxicity to cardiac tissues, its clinical utility is still limited [1]. Congestive heart failure, cardiomyopathy, and electrocardiographic improvements were proven after combined doses of Doxorubicin administration [2]. Free radical-induced myocardial injury, lipid peroxidation [3], mitochondrial damage [4], decreased activity of Na^+–K^+ adenosine triphosphate [5], vasoactive amine release [6], and cellular toxicity have all been reported as potential mechanisms for Dox's cardiotoxic consequences [7]. Oxidative stress [8] and the release of free radicals, such as superoxide anion (O^{2-}) and other reactive oxygen species [9]. Doxorubicin is one of the most effective anti-cancer drugs, but it is limited in clinical use due to cumulative dose-dependent cardiac toxicity, which results in untreatable heart failure (HF) in a large percentage of patients [10]. Doxorubicin works by intercalating into nuclear DNA, impairing protein synthesis, producing reactive oxygen species, and inhibiting topoisomerase II. Doxorubicin is known to target topoisomerase, an enzyme involved in DNA transcription and

replication [11]. Cardiotoxicity is linked to doxorubicin in 3–26% of treated patients, trastuzumab in 2–28%, and sunitinib in 2.7–11% of patients. In a recent retrospective survey, 6.6 percent of breast or haematological cancer patients who received chemotherapy developed heart failure [12]. A few neutralizing specialists against Doxorubicin were researched as phytochemicals [13]. free-extremist foragers as Dexrazoxane (DZR) [14]. Metformin and fibroblast development factor [15]. Notwithstanding, current treatments can't ensure perpetual for insurance. One of their fundamental constraints is that they don't advance myocardium recovery [16]. Therefore, present study was designed to investigate doxorubicin-induced toxicity in albino rat model.

2. Materials and methods

2.1. Experimental Animals

The present study was carried using five to seven weeks- old mature male Sprague Dawly albino rats of an average body weight (200-220 g.). They were apparently normal, healthy animals and obtained from laboratory of Schistosome Biological Supply Program (SBSP) Theodor Bilharz Research Institute. These animals were housed in animal house under regular periods of dark and light i.e. (12-hrs dark and 12 -hrs light) at room temperature. Animals were marked and housed in cages, these cages were cleaned daily. Animals were fed on standard rodent pellet diet manufactured by the Egyptian Company for Oil and Soap as well as some vegetables. Also, the water is supplied during the periods of the experiments. Animal care and housing will be in accordance with the recommendations contained in the DHHS/PHS NIH Publication No. 86–23, 1985, Guidelines for the Care and Housing of Laboratory Animals, or other appropriate guidelines.

2.2. Approval Ethical committee

The study is obtained from the Ethical committee of scientific research (Institutional Research Board “IRB”, faculty of Veterinary Medicine, Banha University.)

2.3. Dose of Doxorubicin

Doxorubicin (DOX) dose used in this study to induce cardiotoxicity was 0.55 and 1.1mg / kg /week for period of 4 weeks. This schedule has been previously found consistent and reproducible for induction of cardiomyopathy weeks [17].

2.4. Experimental Groups

Thirty adult male rats were randomly divided into three groups, each group has ten rats as the following:

Group 1 (Control): received intra-peritoneally (ip) injection of 0.1 ml saline orally for 4 weeks at 24 h. time interval and served as positive control group to evaluate baseline values of various parameters.

Group 2: received 4 injections of 0.55 mg/kg B.W (i.p) of doxorubicin (DOX) every week to reach a cumulative dose of 2.2 mg/kg.

Group 3: received 4 injections of 1.1 mg/kg B.W (i.p) of doxorubicin (DOX) every week to reach a cumulative dose of 4.4/kg.

Two hours post DOX administration rats from all the groups were killed, blood samples were drawn on EDTA (Ethylene Diamine Tetra Acetic Acid) for hematological study.

2.5. Hematological Parameters

Red blood corpuscles (RBCs), White blood cells (WBCs), Differential leukocytes count and hemoglobin concentration (Hb) were measured by using CBC analyzer (Sino thinker. sk9000, U.S).

2.6. Biochemical Parameters

Serum ALT and AST were estimated utilizing the active technique as indicated by Reitman and Frankel [18]., Action of Alkaline phosphatase (ALP) enzyme was determined according to the method of Moss [19], Colorimetric assessment of serum albumin was done according the method of Doumas and his associates [20], Colorimetric determination of total serum bilirubin (TSB) was carried out using Balistreri and Shaw method [21] using available commercial kits obtained from

spectrum, Egypt. Serum urea level was assessed by the colorimetric assay described by Fawcett and Soctt [22], Serum creatinine level was determined by the colorimetric technique by Larsen [23]. Likewise, complete serum bilirubin for all creatures, was resolved With a Hitachi automatic analyzer (Tokyo, Japan).

2.7. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, version 22) was used in data analysis. Data were expressed as mean ± S.E.M One-way analysis of variance (ANOVA) test was used to compare between groups followed by Fisher's least significant difference (LSD) analysis. P values less than 0.05 were considered significant Armitage & Berry [24]. Data were tabulated as it was represented.

3. Results

3.1. Hematological Parameters

Hematological parameters were represented in table (1) The data obtained revealed that RBC.s count showed significant decrease (P<0.05) after 4 weeks of treatment with Dox when compared with the count of control rats. Hemoglobin (Hb) concentration showed a very high significant decrease (P<0.001) Dox treated rats when compared with the compared with the concentrations of control rats. Moreover, HCT % showed a significant decrease (P<0.05) in Dox treated rats when compared with the corresponding values of control rats.

The results of WBC.s count after four weeks of Dox treatment showed the control rats. Otherwise, WBC.s differential count including (lymphocyte, monocyte, neutrophil, monocyte as well as neutrophil) and platelets count showed a significant decrease (P<0.05) when compared with the values of control rats. as shown in table (1).

3.2. Biochemical Parameters

The obtained data from control and Dox treated rats showed that low and high dose of DOX induced hepatic damage as reflected by significantly ($p < 0.05$) elevated serum ALT, TSB, and ALP enzymes activities when compared to control group after 4 weeks. While Albumin recorded a significant decrease when compared to control group as shown in table (2). Serum urea concentration and creatinine levels were elevated (P<0.001) as compared with the corresponding values of control rats.

4. Discussion

The adverse reactions of chemotherapeutic agents limit the dosage and duration of treatment and affect the quality of life have

Table (1): Hematological parameters levels in adult male albino rats treated with Doxorubicin for 4 weeks.

	Normal	DOX (0.55 mg/kg)	DOX (1.1 mg/kg)
RBCs	5.76±1.25	4.89±1.54	4.16±1.63
Hb	16.89±1.22	15.46±1.12	15.12±2.21
WBCs	7.14±0.32	6.11±0.52	5.99±1.14
Neutrophils	18.22±0.23	18.01±0.31	16.21±3.15
Lymphocyte	78.21±2.26	80.16±5.31	83.66±3.11
Monocytes	1.00±0.10	0.89±0.11	0.87±0.21
Eosinophil	3.23±3.68	3.11±2.79	2.82±2.31
Basophils	0.01±0.21	0.01±0.11	0.01±0.07
Platelets	246.90 ± 7.27	211.90 ± 11.00	185.31 ± 19.28

Table (2): Biochemical parameters levels in adult male albino rats treated with Doxorubicin for 4 weeks.

	Control	DOX (0.55 mg/kg)	DOX (1.1 mg/kg)
ALT	78.6±2.3	95.3±3.1	123.7±4.2
ALP	170.5±4.1	254.3±3.6	274.9±4.4
Albumin	3.91±1.1	2.52±1.2	2.13±1.3
TSB	1.11±0.8	1.30±0.6	1.35±0.9
Creatinine	0.89±0.02	1.74±0.31	2.10±0.13
Urea	25.5±1.4	49.7±2.4	71.6±1.9

gained attention of several authors since more than five decades. The present study demonstrated that toxicity induced by DOX. DOX-induced liver and kidney injury by increasing the serum levels of ALT, AST, TSB and BUN. Also DOX-induced hepatorenal toxicity [25]. However, the therapeutic application of DOX has been greatly limited by its dose-dependent toxicity, particularly severe cardiac and hepatic toxicity [26]. DOX poisoning is usually divided into acute toxicity, subacute toxicity and chronic toxicity. Acute poisoning commonly occurs following single use or after a period of treatment, with the most common symptoms including hypotension, arrhythmia and cardiac dysfunction may occur occasionally, and often accompanied by hepatic and renal damage [27,28]. In current study illustrated that DOX toxicity, the single high-dose model is widely used, which provides valuable biological insights into DOX-induced organ injury. DOX-induced organ injury, the present study showed that the significant increase in the activity of AST, ALT, TSB, BUN. These biochemical alterations in the rat model [29&30]. ALT and AST are the enzymes required in the mutual transformation of sugar and protein in the body. ALT predominantly exists in liver cells, and AST is mainly located in myocardial cells; however, the serum level of AST is also increased when the liver is damaged; thus, the increase in the serum level of ALT and AST suggests liver damage. Higher serum aminotransferase activity may be the result of leakage from damaged liver cell membranes following DOX treatment [31]. Therefore, the increase in these biochemical indexes suggest that DOX causes acute damage of the liver and kidney. Since DOX has significant antitumor activity, novel methods to reduce or prevent its detrimental side effects are expected to increase its effectiveness in anticancer therapy [32]. The present study was designed to investigate the toxic effects of DOX in rats. Current data showed that the ALT and ALP levels in serum were significantly elevated. Moreover, creatinine and urea significantly increased following DOX administration. In conclusion, the present study reported the toxic effects of DOX-induced acute toxicity in a rat model.

5. Conclusions

We can conclude that the effect of Doxorubicin-induced toxicity in albino rat model. Showing several biochemical and hematological abnormal effects, further studies are needed to be carried out to protect from the side effect of Doxorubicin.

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التغيرات السمية البيوكيميائية والدموية المحدثة بواسطة الدوكسوروبسون فى الجرذان

ياقوت السنوسى[1]، سامى على حسين1، سيد بكرى [2]، مريم ثابت[1]

1-قسم الكيمياء الحيوية- كلية الطب البيطرى-جامعة بنها-مصر

2-مركز الهندسة الوراثية-جامعة الأزهر-القاهرة-مصر

الملخص العربى :

الدوكسوروبسون عقار مضاد للسطان مستخلص من بكتريا الاستربتوميسيس منذ عام 1970 ونظرا لكفائته كمضاد للسرطان فإنه يستخدم لعلاج العديد من الاورام. ومع هذا فإن له أثار جانبية تحد من إستعماله لذا فقد تم تصميم هذه الدراسة لبيان وتقييم السمية البيوكيميائية والدموية الناتجة عن إستعمال الدوكسوروبسون فى الجرذان البيضاء. تم إستخام ثلاثون جرذا من الذكور ثم تقسيمهم إلى ثلاثة مجموعات (10 في المجموعة الواحدة) الأولى منهم ضابطة والثانية والثالثة ثم تجريعها بجرعات 0.55،1.1 ملجم/كجم/اسبوع. بعد ساعتين من اخر جرعة من جرعات الدوكسوروبسون تم قتل جميع الجرذان وتجميع عينات الدم لعمل التحاليل الدموية والبيوكيميائية ثم تحليل النتائج بواسطة برنامج للتحليل الاحصائى.

أظهرت النتائج التي تم الحصول عليها بعد إجراء التحاليل البيوكيميائية والدموية أن الدوكسوروبسون قد أحدث سمية دموية تتضح في نقص عدد كريات الدمونقص فى تركيز الهيموجلوبين وخلايا الدم البيضاء والصفائح الدموية إذا ما قورنت بالمجموعة الضابطة. ولذلك أحدث الدوكسوربسون سمية كبدية وكلوية ظهرت فى إرتفاع قيم انزيمات الكلى والكبد ونقص فى بروتين الالبيومين وزيادة فى معدل تركيز الكرياتينين. وخلصت الدراسة إلى أنه رغم أن للدوكسوروبسون فوائد علاجية هامة إلا أنه يحدث سمية يجب تجنبها. لذا يجب عمل دراسات مستقبلية لتفادى سمية هذا العقار.