

Effects of Two Different Doses of Zinc Sulfate on Serum Troponin I 3 Enzyme Level and Cardiac Malondialdehyde Contents in Mitoxantrone-Induced Cardiotoxicity in Rats

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Abstract

Mitoxantrone antineoplastic drugs used in the treatment cancers. The mitoxantrone' may cause cardiotoxicity which is irreversible and dose-dependent. Zinc is regarded as a necessary element for cell division and the production of DNA and protein; furthermore, it has a main role in alleviating cardiovascular diseases; and may have protective outcomes in coronary artery disease. The current research is designed to investigate the effects of two different doses of zinc sulfate on mitoxantrone-induced cardiotoxicity in rats. Forty-eight (48) adult Albino rats of both sexes were utilized in this study; the animals were randomly classified into six groups of 8 animals each. **Group I:** Rats were received distilled water (negative control). **Group II:** Orally-administered zinc sulfate (15mg/kg/day) **Group III:** orally-administered zinc sulfate (30mg/kg/day) **Group IV:** Rats Intraperitoneally injected with a mitoxantrone at a dose of (2.5 mg/kg) to reach total cumulative dose of 7.5 mg/kg on day 20 (positive control). **Group V:** Orally-administered zinc sulfate at a dose (15mg/kg/day) and an intraperitoneal injection of mitoxantrone at a dose (2.5 mg/kg) was administered to reach the total cumulative dose of 7.5 mg/kg on day 20. **Group VI:** Orally-administered zinc sulfate at a dose (30 mg/kg/day), and an intraperitoneal injection of mitoxantrone at a dose (2.5 mg/kg) to reach the total cumulative dose of 7.5 mg/kg on day 20. Forty-eight (48) hours after the end of treatment duration (i.e. at day 22nd), each animal was euthanized by diethyl ether and ketamine. Then, after cervical dislocation, blood was obtained by cardiac puncture and then serum was prepared to estimate troponin I 3 enzyme levels, and the heart of each animal was excised for homogenate preparation to estimate of malondialdehyde contents.

It was found that oral administration of zinc sulfate [(15mg/kg/day with total cumulative dose (7.5 mg/kg) of MTXN] (**Group V**), resulted in a non-significant ($P>0.05$) differences in both serum level of troponin I 3 enzyme, and malondialdehyde (MDA) contents in heart tissue homogenate compared to the corresponding level and content in group of rats IP injected with total cumulative dose of 7.5 mg/kg of MTXN (**Group IV**). Moreover, there were significant reductions ($P<0.05$) in serum level of troponin I 3 of rats orally-administered zinc sulfate [(30 mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to the corresponding enzyme levels in serum of **-Group IV** rats that IP injected with total cumulative dose of 7.5mg/kg of MTXN and **-Group V** rats that orally-administered zinc sulfate [(15mg/kg/day with total cumulative dose (7.5 mg/kg) of MTXN]. Furthermore, there were significant reductions ($P<0.05$) in MDA contents in heart tissue homogenate of **Group VI** rats compared to the corresponding contents in heart tissue homogenate of **Group IV** rats.

According to above results it can be concluded that zinc sulfate at a dose of (30 mg/kg/day) (in **Group VI** rats) diminished the cardiotoxicity induced by mitoxantrone as indicated by the reduction of serum troponin I 3 enzyme level but non-significant difference in lipid peroxidation marker (malondialdehyde) compared to (15 mg/kg/day) (in **Group V** rats).

Keywords: Mitoxantrone, Cardiotoxicity, Zinc sulfate, troponin I 3 enzyme, Malondialdehyde contents

تأثير جرعتين مختلفتين من كبريتات الزنك على أنزيم ال troponin I 3 في مصل الدم ومحتوى
المالونداي الدهيد في سمية القلب المستحثة بواسطة عقار ميتوزانترون في الجرذان
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الخلاصة

الميتوزانترون هو علاج مضاد للأورام يستخدم في علاج انواع السرطانات. ان تسمم القلب الناتج عن المايثوزانترون يعد لا عكوس، ويعتمد على الجرعة، وقد يحدث بعد سنوات من العلاج.

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Received: 29/ 7 /2019

Accepted: 12/ 10/2019

يعد الزنك معدنًا أساسيًا لانقسام الخلايا وتوليف الحمض النووي والبروتينين ؛ علاوة على ذلك ، فإن هذا المعدن له دور مهم في تخفيف حالات أمراض القلب والأوعية الدموية و يبدو أن له آثار وقائية في مرض الشريان التاجي واعتلال عضلة القلب. صممت هذه الدراسة لاستقصاء آثار إعطاء جرعتين مختلفتين من كبريتات الزنك على سمية القلب المستحثة بواسطة الميتوزانترون في الجرذان.

تم استخدام ثمانية وأربعون (48) جرذاً بالغاً من كلا الجنسين. تم تقسيم الحيوانات بشكل عشوائي في ست مجموعات من 8 حيوانات لكل منها. **المجموعة الأولى:** الماء المقطر (مجموعة السيطرة). **المجموعة الثانية:** 15 ملغ / كغم / يوم كبريتات الزنك فمويًا. **المجموعة الثالثة:** 30 ملغ / كغم / يوم كبريتات الزنك فمويًا. **المجموعة الرابعة:** حقنة داخل الصفاق بالميتوزانترون بجرعة (2,5 ملغ/كغم) للوصول إلى الجرعة التراكمية الإجمالية البالغة 7,5 ملغ / كغم في اليوم 20. **المجموعة الخامسة:** 15 ملغ/كغم / يوم كبريتات الزنك فمويًا مع إعطاء ميتوزانترون بطريقة الحقن داخل الصفاق بجرعة (2,5 ملغ/كغم) للوصول إلى إجمالي جرعة تراكمية قدرها 7,5 ملغ / كغم في اليوم 20. **المجموعة السادسة:** 30 ملغ/كغم / يوم كبريتات الزنك فمويًا مع إعطاء ميتوزانترون بطريقة الحقن داخل الصفاق بجرعة (2,5 ملغ/كغم) للوصول إلى الجرعة التراكمية الإجمالية البالغة 7,5 ملغ/كغم في اليوم 20. في اليوم الثاني والعشرين من الدراسة و بعد خلع العنق، تم الحصول على الدم عن طريق ثقب داخل القلب وبعد ذلك تم إعداد المصل لتقدير مستويات نشاط انزيم القلب 3 التروبونين؛ كما وتم استئصال قلب كل حيوان من أجل تحضير التجانس لتقدير محتوى المألونداي الديهايد.

وقد أظهرت النتائج ان كبريتات الزنك التي اعطيت فمويًا للجرذان بجرعة (15 ملغ/كغم / يوم) مع الجرعة التراكمية الإجمالية (7,5 ملغ/كغم) من ميتوزانترون داخل الصفاق (المجموعة الخامسة) لم تنتج فرق معنوي ($P > 0.05$) في مستوى نشاط المصل من انزيم التروبونين I3 ومحتوى المألونداي الديهايد في تجانس الأنسجة القلبية مقارنة بمستوى نشاط المصل من الانزيم المقابل ومحتوى المألونداي الديهايد في مجموعة من الجرذان التي تم حقنها داخل الصفاق بجرعة تراكمية إجمالية قدرها 7,5 ملغ/كغم من الميتوزانترون (المجموعة الرابعة)؛ على العكس، كان هناك انخفاض معنوي ($P < 0.05$) في مستوى نشاط المصل لمحتويات انزيم التروبونين I3 و محتوى المألونداي الديهايد في أنسجة القلب المتجانسة من الجرذان التي اعطيت جرعة فموية من كبريتات الزنك (30 ملغ/كغم / يوم) مع الجرعة التراكمية الكلية (7,5 ملغ/كغم) من الميتوزانترون داخل الصفاق (المجموعة السادسة) مقارنة بمستويات نشاط الانزيم ومحتوى المألونداي الديهايد في مجموعة من الجرذان التي تم حقنها داخل الصفاق بجرعة تراكمية إجمالية قدرها 7,5 ملغ/كغم من الميتوزانترون (المجموعة الرابعة).

وبذلك يمكن الاستنتاج ان كبريتات الزنك بجرعة (30 ملغ/كغم / يوم) (جرذان المجموعة السادسة) قللت من نسج القلب المستحثة بواسطة الميتوزانترون من خلال خفض مستوى التروبونين في مصل الدم مع اختلاف غير معنوي في محتوى بروكسيد الدهون (المألونداي الديهايد) مقارنة مع كبريتات الزنك بجرعة 15 ملغ/كغم / يوم (جرذان المجموعة الخامسة).
الكلمات المفتاحية : الميتوزانترون ، سمية القلب ، كبريتات الزنك ، تروبونين القلب ، ومحتوى المألونداي الديهايد.

Introduction

Mitoxantrone (MTXN) is one of the most generally used antineoplastic drug used in treatment of acute leukemia, breast and prostate cancer, lymphoma, and also in the treatment of multiple sclerosis (MS) because of its immunosuppressive features^(1,2). The MTXN-prompted cardiotoxicity is irreversible, and depend on dose. The risk of MTXN-induced cardiac adverse effects can be seriously increased with cumulative dose $> 140 \text{ mg/m}^2$ ⁽²⁾. Clinically, the cardiotoxicity produced by MTXN can cause a reduction in congestive heart failure (CHF) and left ventricular stroke volume⁽³⁾; furthermore, authors stated that the cardio-protective properties of several pharmacologic drugs and a plant have been confirmed by researchers; where, pyridoxine, statins and artichoke have been revealed to be potentially protective against various cardiotoxic agents^(4,5,6).

Zinc (Zn), is one of the main trace elements in the body. It is involved in homeostasis role in oxidative stress (OS). It is required for the metabolic activity of the body's enzymes that involved carbohydrates, fats, proteins the metabolism; and it has an important role in states of cardiovascular diseases (CVDs); and it may have cardio-protective effects in heart disease⁽⁷⁾. The aim of the study is to investigate the effects of two doses of zinc sulphate on MTXN –induced cardiotoxicity.

Materials and Methods

Preparation of drugs solution

Mitoxantrone (MTXN) vial (20mg/10ml) was diluted with 10ml D.W to obtain 1mg /ml to be IP injected at a dose 2.5 mg/kg. Each capsule of zinc sulfate (220mg) was reconstituted in 22ml of D.W to obtain 10mg/ml to be administered by oral gavage at doses 15mg/kg, and 30mg/kg^(8, 9).

Animals

Forty-eight (48) healthy adult Wistar Albino rats of both sexes (24 male and 24 female), three months old, weighing range 150-240 gm were utilized in this research; they were gotten from in the animal House of College of Science, University of Dhi Qar. Rats were maintained in the College of Science, Wasit University under normal conditions of temperature, humidity and under a 12 h light/dark cycle. Rats were supplied commercial pellets and tap water throughout the experiment period. The animals had no manifestation of any illness upon examination. They were left for two weeks without interference for acclimatization. The study was accepted by the Graduate Studies and the Scientific Committees of the College of Pharmacy, University of Baghdad.

Experimental protocol

Healthy rats were randomly allocated into six groups, each containing 8 rats' (4 males and 4 females) as follows:

Group I: Rats received 0.5 ml/day of distilled water (DW) intraperitoneally (IP) for 20 days. This group served as a negative control.

Group II: Rats orally-administered zinc sulfate at a dose of (15mg/kg/day) alone for 20 days.

Group III: Rats orally-administered zinc sulfate at a dose of (30mg/kg/day) alone for 20 days⁽⁸⁾

Group IV: Rats intraperitoneally (IP) injected with a mitoxantrone (MTXN) at a dose (2.5 mg/kg) on day 0, 10, 20 to reach total cumulative dose of 7.5 mg/kg on day 20⁽⁹⁾. This group served as positive control.

Group V- Rats orally-administered zinc sulfate at a dose (15mg/kg/day) for 20 days, with an IP injection of mitoxantrone (MTXN) at a dose (2.5 mg/kg) was administered at the day 0, 10, 20 to reach total cumulative dose of 7.5 mg/kg on day 20.

Group VI- Rats orally-administered zinc sulfate at a dose (30 mg/kg/day) for 20 days, and an IP injection of mitoxantrone (MTXN) at a dose (2.5 mg/kg) was administered at the day 0, 10, 20 to reach total cumulative dose of 7.5 mg/kg on day 20.

Forty-eight (48) hour after the end of the treatment duration (i.e. at day 22nd), each animal was euthanized by diethyl ether and ketamine. Then, after cervical dislocation, two and a half (2.5) ml blood was obtained from each rat by cardiac puncture and then was transferred into a gel tube and left to clot for 30 minutes before centrifugation for 15 minutes at 3000 round per minute (rpm) to obtain serum, which was collected using rubber micropipette, divided into small aliquots in labeled Eppendorf tubes, and kept frozen at -50 °C until analyzed. The serum was utilized for the estimation of troponin I 3; where, the estimation is based on Sandwich-Enzyme-linked immunosorbent assay (ELISA)⁽¹⁰⁾. Serum level of troponin I 3 was expressed as pg/mL. Furthermore, cardiac tissues were obtained to prepare heart tissue homogenates for the determination of MDA contents with the use of phosphate buffer saline (PBS pH 7.4) which also based on ELISA⁽¹¹⁾. The contents of MDA in heart tissue homogenate were expressed as ng/mL.

Statistical Analysis

Statistical analysis was done by Statistical Package for Social Sciences (SPSS) version 24. Results were calculated as mean ± standard error of means (SEM). Comparison among groups was done by using a one-way Analysis of variance (ANOVA). The

statistically significant differences were considered when $P < 0.05$.

Results

Table 1 and figure 1 showed that there were non-significant differences ($P > 0.05$) in serum level of troponin I 3 in group of rats orally-administered zinc sulfate (15mg/kg/day alone for 20 days) (**Group II**) compared to the corresponding enzyme level in serum of negative control rats (**Group I**). Mean±SEM of serum of troponin I 3 levels were 155.09±7.56 vs. 152.47±3.90, respectively. Similarly, table 1 and figure 1 showed that there were non-significant differences ($P > 0.05$) in serum level of troponin I 3 in group of rats orally-administered zinc sulfate (30mg/kg/day alone for 20 days) (**Group III**) compared to the corresponding level in serum of negative control rats (**Group I**). Mean±SEM of serum of troponin I 3 enzyme levels were 150.80±2.966 vs. 152.47±3.90, respectively.

Furthermore, rats IP injected with a total cumulative dose of 7.5mg/kg of MTXN (**Group IV**) (positive control) produced significant elevation ($P < 0.05$) in serum level of troponin I 3 compared to the corresponding level in serum of negative control rats (**Group I**). Mean±SEM of serum cTn-I 3 levels were respectively, 203.71±4.76 and 152.47±3.90.

Meanwhile, table 1 and figure 1 also showed that oral administration of zinc sulfate [(15mg/kg/day with total cumulative dose (7.5 mg/kg) of MTXN] (**Group V**), resulted in a non-significant ($P > 0.05$) difference in serum level of troponin I 3 compared to the corresponding serum level in group of rats IP injected with total cumulative dose of 7.5 mg/kg of MTXN (**Group IV**).

Moreover, there was a significant reduction ($P < 0.05$) in serum level of troponin I 3 in rats orally-administered zinc sulfate [(30 mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to the corresponding levels in serum of rats IP injected with total cumulative dose of 7.5mg/kg of MTXN (**Group IV**). Mean±SEM of serum level of troponin I 3 enzymes were 174.1±2.8 vs. 203.71±4.76 pg/ml, respectively. Table 1 and figure 1.

In addition there were significant reduction ($P < 0.05$) in serum levels of troponin I 3 in rats orally-administered zinc sulfate [(30mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to the corresponding levels in serum of rats orally-administered zinc sulfate [(15mg/kg/day with total cumulative dose (7.5mg/kg) of mitoxantrone (MTXN)] (**Group V**). Mean±SEM of serum troponin I 3 enzymes

were respectively, 174.1±2.8 vs. 195.25±6.7 pg/ml. Table 1 and figure 1.

Table 1. Effects of various treatments on serum cardiac troponin I 3 (cTn-I 3) levels in studied rats' groups

Group/Treatment	Serum cardiac troponin I 3 (cTn-I 3) levels (pg/mL) Mean ± SEM
Group I / Negative control [Distilled water (DW)]	152.47±3.90
Group II /Zinc sulfate (15 mg/kg/day)	155.09±7.56
Group III /Zinc sulfate (30 mg/kg/day)	150.80±2.966
Group IV /Mitoxantrone (MTXN) (7.5 mg/kg) (total cumulative dose)	203.71±4.76*
Group V /Zinc sulfate (15 mg/kg/day) with mitoxantrone (MTXN) (7.5 mg/kg)	195.25±6.7
Group VI /Zinc sulfate (30 mg/kg/day) with mitoxantrone (MTXN) (7.5 mg/kg)	174.1±2.8 #

Data expressed as mean ± standard error of means (SEM).

*: $P < 0.05$: Significant difference compared to negative control rats (**Group I**).

- # $P < 0.05$: Significant difference compared to **Group IV, and Group V**.

- Number of rats in each group=8.

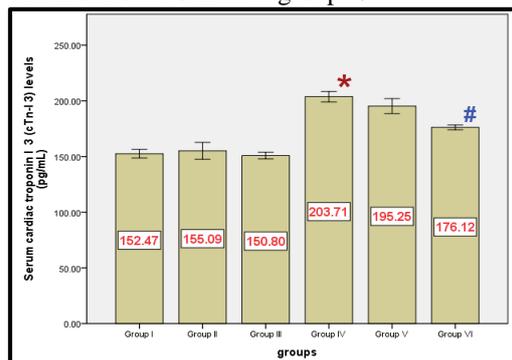


Figure 1. Bar chart showing serum cardiac troponin I 3 (cTn-I 3) level (pg/ml) in various experimental rats' groups.

Group I: Negative control [Distilled water (DW)]; **Group II:** Zinc sulfate (15mg/kg/day); **Group III:** Zinc sulfate (30mg/kg/day); **Group IV:** Mitoxantrone (MTXN) (7.5mg/kg); **Group V:** Zinc sulfate (15mg/kg/day) with Mitoxantrone (MTXN) (7.5 mg/kg); **Group**

VI: Zinc sulfate (30 mg/kg/day) with Mitoxantrone (MTXN) (7.5mg/kg).

*: Significant difference ($P < 0.05$) with respect to the negative control group. (**Group I**)

- # $P < 0.05$: Significant difference compared to **Group IV, and Group V**.

Table 2 and figure 2 showed that there were non-significant differences ($P > 0.05$) in MDA contents in heart tissue homogenate in group of rats orally-administered zinc sulfate (15 mg/kg/day alone for 20 days) (**Group II**) compared to corresponding contents in negative control rats (**Group I**). Mean±SEM of in MDA contents in heart tissue homogenate were 44.48±1.88 and 42.40±.92, respectively. Similarly, there were non-significant differences ($P > 0.05$) in MDA contents in heart tissue homogenate in the group of rats orally-administered zinc sulfate (30 mg/kg/day alone for 20 days) (**Group III**) compared to the corresponding contents in negative control rats (**Group I**). Mean±SEM in MDA contents in heart tissue homogenate levels were 43.91±1.61 and 42.40±.92, respectively.

Furthermore, rats IP injected with a total cumulative dose of 7.5 mg/kg of MTXN (**Group IV**) produced significant elevation ($P < 0.05$) in MDA contents in heart tissue homogenate compared to the corresponding contents in heart tissue homogenate in negative control rats (**Group I**). Mean±SEM of MDA contents in heart tissue homogenate contents were 59.41±4.1 and 42.40±.92, respectively. Table 2 and figure 2.

Moreover, table 2 and figure 2 also showed that administration of zinc sulfate [(15mg/kg/day with total cumulative dose (7.5 mg/kg) of MTXN] (**Group V**), resulted in a non-significant ($P > 0.05$) difference in MDA contents in heart tissue homogenate compared to the corresponding contents in rats IP injected with total cumulative dose of 7.5 mg/kg of MTXN (**Group IV**). Mean±SEM of MDA contents in heart tissue homogenate levels were 54.97±1.67 and 59.41±4.1, respectively.

Furthermore, there were significant reduction ($P < 0.05$) in MDA contents in heart tissue homogenate of rats orally-administered zinc sulfate [(30 mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to the corresponding contents in heart tissue homogenate of rats IP injected with total cumulative dose of 7.5mg/kg of MTXN (**Group IV**). Mean±SEM of MDA contents were 49.91±2.08 and 59.41±4.1 (ng/mL), respectively.

In addition, table 2 and figure 2 showed that there were non-significant differences ($P>0.05$) in MDA contents in heart tissue homogenate of rats orally-administered zinc sulfate [(30mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to MDA contents in heart tissue homogenate of rats orally-administered zinc sulfate [(15mg/kg/day with total cumulative dose (7.5mg/kg) of MTXN] (**Group V**). Mean±SEM of MDA contents in heart tissue homogenate levels were 49.91 ± 2.08 and 54.97 ± 1.6 (ng/mL), respectively.

Table 2. Effects of various treatments on malondialdehyde (MDA) contents in heart tissue homogenate in studied rats' groups

Group	Heart Homogenate MDA (ng/mL) Mean ± SEM
Group I / Negative control [Distilled water (DW)]	42.40 ± 0.92
Group II /Zinc sulfate (15 mg/kg/day)	44.48 ± 1.88
Group III /Zinc sulfate (30 mg/kg/day)	43.91 ± 1.61
Group IV /Mitoxantrone (MTXN) (7.5 mg/kg) (total cumulative dose)	$59.41\pm 4.1^*$
Group V /Zinc sulfate (15 mg/kg/day) with mitoxantrone (MTXN) (7.5 mg/kg)	54.97 ± 1.6
Group VI /Zinc sulfate (30 mg/kg/day) with mitoxantrone (MTXN) (7.5 mg/kg)	$49.91\pm 2.08^\#$

Data expressed as mean ± standard error of means (SEM).

*: $P<0.05$: Significant difference compared to negative control rats (**Group I**).

- # $P<0.05$: Significant difference compared to **Group IV**.

- Number of rats in each group=8

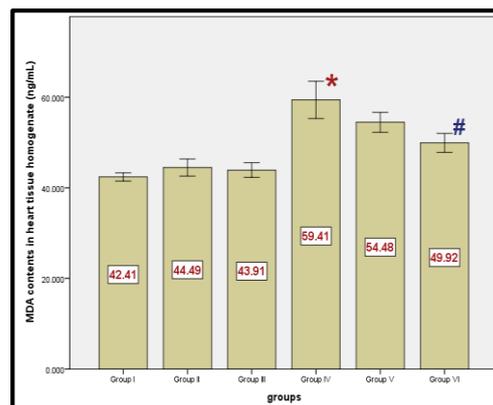


Figure 2. Bar chart showing MDA contents in heart tissue homogenate in various experimental rats' groups.

Group I: Negative control [Distilled water (DW)]; **Group II:** Zinc sulfate (15mg/kg/day); **Group III:** Zinc sulfate (30mg/kg/day); **Group IV:** Mitoxantrone (MTXN) (7.5mg/kg); **Group V:** Zinc sulfate (15mg/kg/day) with Mitoxantrone (MTXN) (7.5 mg/kg); **Group VI:** Zinc sulfate (30 mg/kg/day) with Mitoxantrone (MTXN) (7.5mg/kg).

- *: Significantly different ($P<0.05$) with respect to the negative control group. (**Group I**)
 - # $P<0.05$: Significant difference compared to **Group IV**.

Discussion

Mitoxantrone (MTXN) is an antineoplastic agent used to treat several types of cancers and on multiple sclerosis (MS); such drug showed a high incidence of cardiotoxicity, which was reported to depend on several factors; where, age and cumulative dose being two important factors ⁽¹²⁾.

In the current study, IP injection of MTXN can cause destruction of the myocardial cell membrane, which becomes more permeable; thus, serum troponin I 3 level were significantly elevated ($P<0.05$) compared to the corresponding levels in negative control rats' group (table 1 and figure 1). This comes in tune with a study of others ⁽¹³⁾.

Biomarkers are molecules which occur naturally and can be used as an indicator of a particular disease state or some other physiological state of an organism. Troponins were medium-sized proteins which have an important role in regulating the cardiac muscle contractile elements (myosin and actin) ⁽¹⁴⁾. Furthermore, troponins are well-established biomarkers for the discovery of heart damage in both human and animals; where, levels may increase after 2-3 hours; and serum levels of cTnI and cTnT components of the troponin complex of muscle cells, are of importance in the diagnosis of myocardial damage in several conditions, including acute myocarditis ^(15,16, 17).

The serum levels of troponin I 3 to detect myocardial destruction induced by MTXN was not previously estimated; but the study performed by Herman EH, *et al* at 2001⁽¹⁸⁾ and by the determination of troponin T levels showed that there were high levels of the biomarker in rats with chronic doxorubicin- and MTXN-induced cardiotoxicity .

Thus, to our knowledge the current research is the first to estimate the serum level of troponin I 3 enzyme in MTXN-induced cardiotoxicity; thus, we did not have a chance to compare results obtained from this study with others concerning this respect.

The results of the present study also showed that oral administration of zinc sulfate [(15mg/kg/day with total cumulative dose (7.5 mg/kg) of MTXN] (**Group V**), resulted in a non-significant ($P>0.05$) difference in serum level of troponin I 3 compared to the corresponding serum level in group of rats IP injected with total cumulative dose of 7.5 mg/kg of MTXN (**Group IV**). Moreover, there were significant reduction ($P<0.05$) in serum level of troponin I 3 of rats orally-administered zinc sulfate [(30 mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to the corresponding levels in serum of rats IP injected with total cumulative dose of 7.5mg/kg of MTXN (**Group IV**) (table 1 and figure 1).

Zinc is a vital element in preserving the normal structure and physiology of cells. It appears to have protective effects in heart diseases⁽¹⁹⁾. Furthermore, authors reported ischemia and infarction may lead to release of zinc from proteins and cause myocardial damage. In such states, replacing with zinc has been shown to recover cardiac function and prevent further destruction⁽²⁰⁾. Moreover, authors reported that rodents administered zinc salts during reperfusion has been stated to decrease arrhythmias and improve myocardial function⁽¹⁹⁾.

Additionally, to assess if MTXN is able to initiate or enhance lipid peroxidation, the most known secondary product of lipid peroxidation, MDA, was determined; where, it is one of the best indicators of OS⁽²¹⁾.

Results of this study exhibited that the cardiac tissue MDA content was significantly elevated ($P<0.05$) in MTXN-treated rats (**Group IV**, positive control) compared to the corresponding cardiac contents in negative control (**Group I**) rats (table 2 and figure 2); these results are in line with the work of others. The increment in the product of lipid peroxidation (MDA) may be considered as a causative factor in MTXN-induced acute cardiotoxicity^(22, 23)

Furthermore, results of this study showed that that oral administration of zinc sulfate [(15mg/kg/day with total cumulative dose (7.5 mg/kg) of MTXN] (**Group V**), resulted in a non-significant ($P>0.05$) difference in MDA contents in heart tissue homogenate compared to the corresponding contents in heart tissue in group of rats IP injected with total cumulative dose of 7.5 mg/kg of MTXN (**Group IV**). Moreover, there were significant reduction ($P<0.05$) in MDA contents in heart tissue homogenate of rats orally-administered zinc sulfate [(30 mg/kg/day) with total cumulative dose (7.5mg/kg) of MTXN] (**Group VI**) compared to the corresponding MDA contents in heart tissue homogenate of rats IP injected with total cumulative dose of 7.5mg/kg of MTXN (**Group IV**).

Authors revealed that Zn, a necessary metal that is integral to the activity of many metalloenzymes required for cellular functions; and it has critical, anti-atherogenic, anti-inflammatory and antioxidant actions to protect against various oxidative stresses

Moreover, the authors describe that there is a strong basis to consider the strategy of Zn supplementation to cardioprotect the heart against MI⁽²⁴⁾. Furthermore, Asri-Rezaei Siamak *et al* (2017) observed that, zinc sulfate administration could protect against lipid peroxidation, due to anti-oxidative effect that showed by a reduction in MDA contents in rats. Furthermore, others reported that zinc-induced a very efficient antioxidant in scavenging various free radicals [metallothionein (MT)] synthesis in the rat heart which in turn may alleviate diabetic cardiotoxicity⁽⁸⁾. In addition, at 2015 Hala Attia *et al* reported that addition of Zn to glibenclamide can significantly reduce MDA in cardiac tissues compared to glibenclamide alone; this can support the powerful antioxidant effect of Zn and potential cardio-protection from oxidative damage⁽²⁵⁾.

Conclusion

Zinc sulfate at a dose of (30 mg/kg/day) (in **Group VI** rats) diminished the cardiotoxicity induced by mitoxantrone as indicated by the reduction of serum troponin I 3 enzyme level but non-significant difference in lipid peroxidation marker (malondialdehyde) compared to (15 mg/kg/day) (in **Group V** rats).

Acknowledgments

This article was abstracted from a Ph.D. thesis submitted to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. Authors deeply indebted to Dr. Saleema S. Salman, the University of Baghdad, College of Pharmacy for her valuable assistance in statistical analysis.

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