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Evaluation the antioxidant effect of chromium picolinate in doxorubicin induced cardiotoxicity in a rat model

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ABSTRACT: Doxorubicin (DOX) is a potent antineoplastic drug used to treat many types of human tumor. The long-term adverse effect is cardiomyopathy. Chromium is an essential trace element mostly used to regulate glucose levels and enhance the response to insulin, especially in diabetes. Current study aimed to evaluate the cardioprotective effect of chromium picolinate against doxorubicin-induced cardiotoxicity in 28 male rats divided into four groups. Group I (Control group): received distilled water orally for 8 days. Group II (Doxorubicin group): received distilled water orally for 7 days, followed by a single doxorubicin dose (25 mg/kg) intraperitoneally. Group III (Chromium 2 mg): received chromium picolinate at a dose (2 mg /kg) orally for 7 days, followed by a single doxorubicin dose (25 mg/kg) intraperitoneally. Group IV (Chromium 4 mg): received chromium picolinate at a dose (4 mg /kg) orally for 7 days, followed by a single doxorubicin dose (25 mg/kg) intraperitoneally. Levels of lactate dehydrogenase (LDH) and serum troponin were assessed in the sera of all groups by ELISA technique while the cardiac homogenate used to assess malondialdehyde (MDA) by ELISA technique and superoxide dismutase (SOD) by RT-qPCR method and the results indicated that the co-administration of chromium picolinate at dose (2mg/kg) and (4mg/kg) caused a significant decrease in cardiac LDH and MDA and significant elevation in SOD level in groups III and IV compared to group II This current research indicated that Chromium picolinate have a potential role in reducing cardiac injury and oxidative stress in patients treated with doxorubicin.

KEYWORDS: Cardiotoxicity; chromium picolinate; lactate dehydrogenase; superoxide dismutase; serum troponin.

1. INTRODUCTION

Doxorubicin (DOX) is a powerful antineoplastic drug used to treat many different human tumors. Long-term treatment with DOX causes a cumulative dose dependent cardiomyopathy in which the cardiac tissues are injured, leading to myocardial dysfunctions [1]. The cardiotoxicity is attributed to generation of free radical, stimulation of lipid peroxidation, mitochondrial dysfunction, and subsequent modification of cellular membrane integrity [2]. Oxidative stress is supposed to be the leading cause of DOX-induced cardiotoxicity because the cardiac tissues lack sufficient antioxidant mechanisms such as glutathione, catalase, superoxide dismutase (SOD), and vitamin E. This makes it more susceptible to oxidative stress and reactive oxygen species ROS generation [3]. The redox cycle is associated with the presence of the quinone moiety, allowing DOX to act as an electron acceptor [4].The reduction of one electron of quinone moiety via NADPH and cytochrome P450 reductase converted DOX into a semiquinone radical which reacts with oxygen (O_2), resulting in generation the superoxide radical $O_2^{\bullet -}$ and reproduction of the quinone form [4]. This redox cycle is intermediated by reduced nicotinamide adenine dinucleotide NADH or NAD(P)H dependent enzymes. The dismutation of $O2^{\bullet -}$ to hydrogen peroxide (H_2O_2) is initiated by SOD [5]. H2O2 is a relatively stable and non-toxic molecule, which is removed by glutathione peroxidase or catalase under physiological conditions. However, H_2O_2 and $O_2^{\bullet -}$ can generate highly toxic and reactive hydroxyl radicals ($OH^{\cdot}$) that react with any oxidizable substance, causing injury to many types of macro-molecules, such as nucleic acids, lipids, and proteins. However, H_2O_2 can undergo the Fenton reaction in the presence of ferrous ion (Fe^{2+}), producing the highly reactive hydroxyl radicals [5].

Treatment with DOX caused structural alteration in the mitochondria, resulting in the depletion of ATP generation. The interaction between DOX and a membrane phospholipid, cardiolipin that presents in the inner membrane of mitochondria in cardiomyocytes, appears to be affected by DOX induced cardiotoxicity. The

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anionic charge of cardiolipin binds irreversibly with the cationic charge of DOX, resulting in the formation of doxorubicin cardiolipin complex causing the mitochondrial dysfunction. The DOX cardiolipin complex affects the role of cardiolipin in the chain of electron transport, with the consequent inhibition of many enzymes, such as cytochrome c oxidase and cytochrome c oxidoreductase [6].

Chromium (Cr) is an essential trace element mostly used to regulate glucose levels and enhance the response of body to insulin, especially in diabetes [7] . This mineral has two different forms: a toxic hexavalent form and an organic trivalent form. Chromium supplements often contain Chromium in its trivalent state, coupling with ligands such as picolinic acid, to form a compound known as chromium picolinate [8]. Chromium (Cr+3) is commonly known for its potential role in regulating glucose levels by the activation of apochromodulin to chromodulin [9]. Indeed, chromium picolinate has been found to have valuable effects on cardiovascular diseases by lowering cholesterol levels and blood pressure, as well as anti-inflammatory and antioxidant ability [10]. It also has the ability to cause physical changes such as improved body composition and training performance, and to modulate behavioral patterns such as depression and anxiety [11]. According to the results of recent studies, trivalent chromium acts as a cofactor in the activation of insulin as a hormone that mediates the metabolism of lipids, proteins and carbohydrates, and thus can play a role in the prevention and treatment of T2DM and CVDs [12].

The current study is designed to evaluate the cardioprotective and antioxidant effect of chromium picolinate in doxorubicin induced cardiotoxicity in a rat model by measuring the levels of troponin as an indicator of cardiac toxicity and by determining the levels of LDH as an indicator of cardiac damage and oxidative stress marker that include malondialdehyde (MDA) and SOD and assess the effect of the administration of chromium picolinate on these markers.

2. RESULTS

2.1. Effect of Chromium picolinate on serum Lactate dehydrogenase (LDH) levels

The data analysis in Table1 indicated a significant elevation in the serum LDH levels in group II and group III comparing to group I ($P<0.05$). At the same time, there is a significant decrease in LDH can be observed when comparing both group III and group VI with group II ($P<0.05$). Moreover, there was a significant difference when group III was compared to group IV ($P<0.05$). On the other hand, the levels of LDH showed to be reduced in group IV to levels comparable to those of controls.

Table 1. The Effect of Chromium picolinate on serum levels of LDH induced by doxorubicin in rats

	GROUPS	LDH
I.	**Negative control group**	27.99 ± 4.25
II.	**DOX group**	91.78 ± 1.37*
III.	**Chromium 2mg/Kg+ DOX**	76.95 ± 2.95*#a
IV.	**Chromium 4mg/Kg + DOX**	29.41 ± 4.15#b

Data was expressed as Mean± STD.
Superscript (*) shows a significant difference when comparing to group I ($P<0.05$).
Superscript (#) shows a significant difference when comparing to group II ($P<0.05$).
Values with small letter superscripts (a, b) between group III and group IV are considered significantly different ($P<0.05$).

2.2. Effect of Chromium picolinate on serum Troponin levels

The data analysis in illustrated in Table 2 indicated a significant increase in the serum troponin I levels in group II and group III comparing to group I ($P<0.05$). At the same time, there is a significant decrease in serum troponin I can be observed when comparing both group III and group VI with group II ($P<0.05$). Moreover, there was a significant difference when group III was compared to group IV ($P<0.05$). In contrary, group IV showed a non-significant difference ($p>0.05$) in the levels of troponin when compared to controls' levels.

2.3. Effect of Chromium picolinate on cardiac MDA levels

The data analysis in illustrated in Table 3 indicated a significant elevation in the cardiac MDA levels in group II and group III comparing to group I ($P<0.05$). At the same time, there is a significant decrease in cardiac MDA levels can be observed when comparing both group III and group VI with group II ($P<0.05$). Moreover, there was a significant difference when group III was compared to group IV ($P<0.05$). In a manner similar to

that obtained with LDH and troponin, MDA in group IV showed reduced levels which is non-significantly differ from those of controls.

Table 2. The Effect of Chromium picolinate on serum troponin induced by doxorubicin in rats

GROUPS		Troponin I
I.	**Negative control group**	114.97 ± 25.53
II.	**DOX group**	459.40 ± 18.16*
III.	**Chromium 2mg/Kg+ DOX**	253.79 ± 12.31*#a
IV.	**Chromium 4mg/Kg + DOX**	137.99 ± 13.94#b

Data was expressed as Mean± STD.
Superscript (*) shows a significant difference when comparing to group I (P<0.05).
Superscript (#) shows a significant difference when comparing to group II (P<0.05).
Values with small letter superscripts (a, b) between group III and group IV are considered significantly different (P<0.05).

Table 3. The Effect of Chromium picolinate on cardiac levels of MDA induced by doxorubicin in rats

GROUPS		MDA
I.	**Negative control group**	4.71 ± 1.06
II.	**DOX group**	49.97 ± 5.04*
III.	**Chromium 2mg/Kg+ DOX**	12.36 ± 0.39*#a
IV.	**Chromium 4mg/Kg + DOX**	7.41 ± 1.34#b

Data was expressed as Mean± STD.
Superscript (*) shows a significant difference when comparing to group I (P<0.05).
Superscript (#) shows a significant difference when comparing to group II (P<0.05).
Values with small letter superscripts (a, b) between group III and group IV are considered significantly different (P<0.05).

2.4. Effect of Chromium picolinate on SOD m RNA expression

The data analysis in illustrated in Table 4 indicated a significant decrease in the gene expression level of SOD in group II and group III as compared to group I (P<0.05), at the same time, there is significant elevation in gene expression levels of SOD can be observed when compared both group III and group VI with group II (P<0.05) .Moreover, there was no significant difference when group III was compared to group IV(P>0.05). Levels of SOD showed a significant reduction in group IV to reach levels similar to those of controls.

Table 4. The Effect of Chromium picolinate on (SOD) m RNA expression induced by doxorubicin in rats

GROUPS		SOD (folds)
I.	**Negative control group**	1.05 ± 0.32
II.	**DOX group**	0.007 ± 0.003*
III.	**Chromium 2mg/Kg+ DOX**	0.33 ± 0.15*#
IV.	**Chromium 4mg/Kg + DOX**	0.74 ± 0.45#

Data was expressed as Mean± STD.
Superscript (*) shows a significant difference when comparing to group I (P<0.05).
Superscript (#) shows a significant difference when comparing to group II (P<0.05).
Values with small letter superscripts (a, b) between group III and group IV are considered significantly different (P<0.05).

3. DISCUSSION

Doxorubicin is one of the most cytotoxic agents used in the experimental study of cardiac diseases. The predominant occurrence of cardiac failure and cardiomyopathy is the primary adverse effect associated with the administration of DOX [13]. Chronic exposure to doxorubicin can lead to structural alterations in the cardiac tissue, including fibrosis and hypertrophy, which compromise cardiac function. It increases the high risks of mortality and morbidity and reduces the clinical advantages of this important medication [14] and for all abovementioned information, the present wok used DOX to induce cardiotoxicity to evaluate the cardioprotective and antioxidant activity of chromium picolinate in DOX-induced cardiotoxicity in a rat model.

To evaluate cellular damage and membrane leakage in cardiac tissue, the integrity of the plasma membrane is often assessed by monitoring the activities of cytoplasmic enzymes such as LDH and Troponin in the blood [15]. Lactate dehydrogenase is an enzyme that facilitates the conversion of lactate to pyruvate

intracellularly, hence playing a vital part in cellular respiration and the generation of energy. There is a potential association between LDH and doxorubicin in cancer treatment [16]. In this current study, DOX administration to rats in group II led to a significant elevation of LDH levels due to cardiac tissue damage and oxidative stress causing leakage of this enzyme into circulation in comparison to group I (control group). These results were similar to those of other previous research and showed the occurrence of cardiac injury after doxorubicin treatment [17]. Furthermore, pre-treatment with chromium picolinate in groups III and IV significantly reduced LDH levels compared to DOX group II, reflecting decreased myocardial damage and restoration of cardiac functions which is consistent with a recently published study conducted by Abdel-Hady on hypoxic rat and demonstrated that chromium picolinate supplementation increased body weight, lowered blood pressure, reduced ventricular hypertrophy and significantly improved the cardiac performance [18].

Troponin is a calcium regulatory protein used for contractile function in cardiac and skeletal tissues. This protein is extended regularly along the length of thin filaments forming complex with actin and tropomyosin. Troponin is consisted of three various subunits, troponins T, I, and C, which are responsible for essential functions, such as the binding of calcium (troponin C), the inhibiting interaction of actomyosin (troponin I), and the binding to tropomyosin (troponin T) (19) . When cardiac tissues are damaged, troponin is released into blood. Elevated levels of troponin are highly specific indicators of cardiac injury, particularly troponin I and T (20). In this current study, DOX administration to rats in group II led to a significant elevation of troponin I levels due to cardiac tissue damage and oxidative stress causing leakage of this protein in circulation in comparison to group I (control group). These results were similar to those of other previous research and showed the occurrence of cardiac injury after doxorubicin treatment [21,22]. Furthermore, pre-treatment with chromium picolinate in groups III and IV significantly reduced serum troponin I levels compared to DOX group II, reflecting decreased myocardial damage and restoration of cardiac functions. These results indicated that chromium picolinate have a cardio protective effect against cardiotoxic effects of DOX in reducing the activities of cardiac biomarkers through its antioxidant properties. These findings are in agreement with previous study conducted in Iraq and demonstrated that the level of chromium was decreased in patients with myocardial infarction who diagnosed with a high troponin level and showed that the level of troponin was inversely related to the chromium levels which may indicate the cardioprotective role of chromium against various cardiac injuries [23]. Moreover, another study also demonstrated that orally administered Chromium picolinate was associated with improved recovery of coronary circulation and myocardial performance following acute ischemia reperfusion insult which is owned to the role of Chromium Picolinate in augmenting NO-mediated endothelium-dependent vasorelaxation [24]

In this current study, administration of DOX in rats led to significant increase in lipid peroxidation which was indicated by significant elevation in MDA levels and was also accompanied by a significant depletion of antioxidant enzymes such as SOD.

Malondialdehyde (MDA) or reactive aldehyde is one of the end of lipid peroxidation products in the cells which is potentially important indicator of the antioxidant status and oxidative stress in cancer (24). An increase in free radicals results in over generation of MDA which reacts with thiol and amino groups resulting in denaturation of proteins and DNA damage [25]. In this current study, DOX administration to rats in group II led to a significant elevation of MDA levels due to cardiac tissue damage and oxidative stress in comparison to group I (control group). These results were similar to those of other previous research and showed the occurrence of cardiac injury after doxorubicin treatment [26,27]. Furthermore, pre-treatment with chromium picolinate in groups III and IV significantly reduced MDA levels and attenuating DOX mediated cardiac lipid peroxidation as compared to DOX group II.

Superoxide dismutases (SODs) are a group of metalloenzymes, forming first line of antioxidant defense against oxidative stress (28). These enzymes alternately stimulate the dismutation of free radical superoxide anion (O2-) into hydrogen peroxide (H2O2) and molecular oxygen, decreasing level of superoxide anion which caused the cardiac injury at high concentration [28]. This reaction is joined by alternate oxidation reduction of metal ions such as Copper, Zinc, Iron, Manganese, and Nickel present in the active site of SODs [29] . In this current study, DOX administration to rats in group II led to a significant decrease of SOD levels due to cardiac tissue damage and oxidative stress in comparison to group I (control group). These results were similar to those of other previous research and showed the occurrence of cardiac injury after doxorubicin treatment [29,30]. Furthermore, pre-treatment with chromium picolinate in groups III and IV significantly increase SOD levels compared to DOX group II. These results indicated that chromium picolinate have a cardio-protective effects against cardiotoxic effects of DOX by attenuating cardiac lipid peroxidation MDA and increasing the activity of antioxidant enzymes such as SOD.

The results obtained in the present work showed that the use of chromium picolinate in a dose of 4mg/Kg can lead to significant reduction in the levels of LDH, troponin and MDA to levels comparable to those of controls which may prove the cardioprotective and anti-oxidant effect of this dose against DOX-induced cardiac injury, and also showed a significant improvement in the levels of SOD to a levels similar to those of controls which also confirm the antioxidant role of this dose against DOX-induced cardiac injury. These findings consistent with several previous studies, reported that chromium causes a reduction in cholesterol levels and blood pressure, as well as anti-inflammatory and antioxidant abilities, as it has a role in scavenging reactive oxygen species through the activation of anti-oxidant enzymes [10, 31]. Another study also reported that chromium supplementation played a significant role in alleviating the cardiac toxicity induced by daunorubicin in patients suffering from cancer as they demonstrated that the levels of aspartate transaminase (AST), LDH and creatinine kinase (CK) were reduced with the use of chromium for mice with daunorubicin-induced cardiotoxicity [32]. Moreover, previous literatures reported that low plasma chromium levels were observed in patients with coronary artery disease and demonstrated that chromium deficiency leads to impaired lipid and glucide metabolism and results in high circulating insulin levels. This, in turn, may give rise to vascular lesions, lower HDL/LDL ratios and increased levels of atherogenic LDL. Chromium supplementation, on the other hand, could have beneficial effects on hypertension, bioenergetics, action potentials (arrhythmias) and obesity, and perhaps minimise the harmful consequently of oral contraceptive use [33].

4. CONCLUSION

In conclusion, the outcome of this current work indicated that chromium picolinate has a cardio protective impact against cardiotoxicity induced by doxorubicin. Chromium picolinate has been found to play an important role as a potential anti- oxidant agent in chemotherapy. It could be used as a cytoprotective agent in doxorubicin treated patients as it showed a significant cardioprotective and antioxidant effect which elucidated clearly in the significant reduction in the levels of LDH, troponin and MDA and the significant improvement in the levels of SOD.

5. MATERIALS AND METHODS

5.1. Materials

In this current study, Doxorubicin HCL and Chromium picolinate were used. Doxorubicin HCL was acquired from Pfizer laboratories in USA, whereas chromium picolinate was obtained from Source Naturals in the United States.

5.2. Experimental design

The study was approved by the scientific and ethical committees of the College of Pharmacy University of Baghdad. Approval Number: RECAUBCP221122023K in 21/12/2023. Wister male rats weighing (160-200) grams were brought and maintained in the Baghdad University College of Pharmacy's animal house. The rats were kept at standard temperatures, humidity, and light/ dark cycles. The rats were housed in plastic cages, seven per cages.

28 Rats used in the work were divided into 4 groups:

1. Group I- (Control group): Rats received distilled water orally for eight days.

2. Group II (DOX group): Rats that received distilled water orally for 7 days. On the eighth day, rats were injected with doxorubicin as a single dose (25 mg/kg) IP [32].

3. Group III (Chromium 2 mg): Rats that received Chromium picolinate at a dose (2 mg /kg) orally for 7 days. On the eighth day, rats were injected with doxorubicin in a single dose (25 mg/kg) IP [32,33].

4. Group IV (Chromium 4 mg): Rats that received Chromium picolinate at a dose (4 mg /kg) orally for 7 days. On the eighth day, rats were injected with doxorubicin in a single dose (25 mg/kg) IP [32, 34].

After 24 hours from DOX administration, whole blood was collected from the jugular vein under diethyl ether anesthesia. Then, the animals were euthanized on the ninth day by cervical dislocation. Then, cardiac tissues were separated for examination [35].

5.3. Preparation of serum samples

Animals' blood was drawn from the jugular vein (near the throat or neck) under diethyl ether anaesthesia [36, 37]. Whole blood was collected in a serum separator tube at room temperature for 30 minutes for clotting. Then, centrifuging for 15 minutes at 3000 rpm to obtain serum. The supernatant was then transferred as 250µl aliquots into appropriately labelled micro-centrifuge tubes and maintained at −20°C until use for the estimation of LDH and serum troponin by ELISA technique according to manufacturer instruction (Cloud Clone Corp) [38, 39].

5.4. Preparation of cardiac tissue homogenate

After the animals have been euthanized, the heart was excised rapidly, and cleaned with extremely cold PBS (phosphate buffered saline) (pH=7.4, 4˚C) to remove excessive blood. Then, heart was blotted on filter paper, weighed. For each animal, the left ventricle was used to make the homogenate of cardiac tissue by cutting down this tissue into fine pieces. Then, 10% cardiac homogenate was prepared by adding 0.9 ml of PBS and 0.1 g of the cardiac tissue into a 2 ml micro-centrifuge tube, followed by homogenization with a tissue homogenizer for 1 minute at 4˚C. Later, tissue homogenate was centrifuged in a refrigerated centrifuge for 10 minutes at 10,000 rpm. Then, the resultant supernatant was collected in a precooled 5 ml test tube, mixed quickly by vortex mixer, and transferred as 200µl aliquots into precooled, appropriately labelled micro-centrifuge tubes [40, 41]. All samples were then maintained at −20˚C until used for estimation of MDA by ELISA technique and SOD by RT-qPCR method.

5.5. Gene Expression analysis

"The Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)" method was utilized to estimate gene expression level of SOD relative to the housekeeping gene GAPDH as a reference gene in cardiac tissue samples. The assay briefly involved the cardiac tissue homogenate with "TRIzo"l was used for the isolation of total RNA using "TransZol Up Plus RNA Kit (TransGen ،biotech)"; subsequently، a complementary DNA (cDNA) synthesis was performed using "the EasyScript® one-step gDNA removal and cDNA synthesis (TransGen ،biotech)". The mRNA expression levels were performed by "SYBR Green Supermix (TransGen ،biotech) " with GAPDH as housekeeping gene [42, 43]. The sequence of primers for GAPDH and SOD showed in table 5.

Table 5. The sequence of the primers used in this study

Primer	Sequence 5′→3′ direction
GAPDH F	CCATCAACGACCCCTTCATT
GAPDH R	CACGACATACTCAGCACCAGC
SOD F	AGGGCGTCATTCACTTCGAG
SOD R	CTCTCTTCATCCGCTGGACC

5.6. Statistical analysis

The numeric data existent in this study were expressed as "mean ± standard deviation (STD)". The statistical analysis was done by "the Statistical Package for Social Sciences software (SPSS)" version 25. The differences between the mean values were determined by ANOVA. The significance threshold of $P<0.05$ was employed to detect statistical significance. If the estimated P-value is less than 0.05, it shows statistical significance [44,45].

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