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Research Article

FEBUXOSTAT MODULATES OXIDATIVE AND APOPTOTIC PATHWAYS IN ACUTE DOXORUBICIN-INDUCED CARDIOTOXICITY: AN EXPERIMENTAL ANIMAL MODEL STUDY

HAYDER M AL-KURAISHY[1], ALI I AL-GAREEB[2], HANY AKEEL NAJI[3]

[1]Department of Clinical Pharmacology, Medicine and Therapeutic, Medical Faculty College of Medicine, Al-Mustansiriya University, Baghdad, Iraq. [2]Department of Clinical Pharmacology, Medicine and Therapeutic, Medical Faculty College of Medicine, Al-Mustansiriya University, Baghdad, Iraq. [3]Department of Pharmacology . Email: hayderm36@yahoo.com

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ABSTRACT

Objectives: Doxorubicin is one of the most important and powerful anticancer drugs, the most pronounced limitation for its use is toxicity on normal cells. Mechanism of doxorubicin-induced cardiotoxicity (DIC) is multifactorial and complex, including direct DNA damage, formation of free radicals, interference with DNA repair, and activation of immune reactions. Febuxostat is a non-purine-selective xanthine oxidase inhibitor decrease the production of uric acid. The aim of the present study was to evaluate the influence of febuxostat on doxorubicin-induced acute cardiotoxicity in rats regarding oxidative stress and antiapoptotic effects.

Methods: A total of 30 Sprague Dawley male rats were used which subdivided into three groups: Group I (negative control group) received normal saline for 10 days, Group II (positive control group) received normal saline plus single dose of doxorubicin (15 mg/kg, IP), and Group III (treated group) received febuxostat (10 mg/kg, po), for 10 successive days plus single dose of doxorubicin (15 mg/kg, I.P.). Serum brain natriuretic peptide (BNP), cardiac troponin I (cTn-I), caspase-3, glutathione peroxidase (GSH-Px), lipid peroxidase (LPO), malondialdehyde (MDA), and tumor necrosis factor alpha were estimated by ELISA kit method.

Results: Febuxostat administration before doxorubicin led to significant decrease on cardiac troponin, caspase-3, and elevation in GSH-Px levels significantly $p<0.05$. While the effects of febuxostat on BNP, LPO, MDA, tumor necrosis-alpha were insignificant $p>0.05$ compare to doxorubicin.

Conclusion: Febuxostat attenuates DIC through modulation of antioxidant, anti-inflammatory, and antiapoptotic biomarkers.

Keywords: Doxorubicin, Febuxostat, Cardiotoxicity.

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INTRODUCTION

Doxorubicin is one of the most important and prominent anticancer drugs. Doxorubicin has the ability to fight rapid dividing cells and to slow the progression of the many malignant diseases [1]. However, clinical usefulness of doxorubicin is limited by its cumulative, dose-dependent progressive cardiotoxicity [2].

It has been categorized three distinct types of doxorubicin-induced cardiotoxicity (DIC), including acute (within days), early-onset chronic (within weeks), and chronic progressive cardiotoxicity (within months-years) after drug administration. Acute DIC may occur during doxorubicin initiation or immediately later which appears as temporary ECG abnormalities, while chronic DIC is presented as congestive heart failure [3].

Different molecular mechanisms are involved in DIC which is multifactorial and complex, include direct DNA damage, formation of free reactive oxygen radicals, interference with DNA repair, alterations of cellular calcium homeostasis, mitochondria injury, lysosomal changes, and apoptosis [4,5]. The heart is particularly sensitive to the oxidative damage due to low levels of antioxidant enzymes, large density of mitochondrial, and high rate of oxygen consumption [6]. Moreover, repeated doxorubicin administration promotes nitric oxide (NO) production in the myocardium through induction of inducible NO synthase expression. The cardiotoxic effect of NO is due to the formation of reactive nitrogen species which attack cellular biomolecules, causing energy imbalance and death [7].

Febuxostat is a non-purine-selective xanthine oxidase inhibitor, blocks the active site of xanthine oxidase. Hence, febuxostat decreases the production of uric acid [8]. Krishnamurthy *et al.* study demonstrated a significant effect of febuxostat on attenuation of DIC through reduction of cardiac injury biomarkers and activation of endogenous antioxidant capacity as febuxostat improves diastolic pressure with significant positive inotropic and lusitropic effects [9]. These findings give a clue that febuxostat could produce a cardioprotective due to its antioxidant effect.

Therefore, the aim of the present study was to evaluate the influence of febuxostat on doxorubicin-induced acute cardiotoxicity in rats regarding oxidative stress and antiapoptotic effects.

METHODS

A total of 30 Sprague Dawley male rats were used in this study, which purchased from International Center for Cancer and Medical Genetic Researches. Rat's body weight ranged from 150 to 250 g with the age range of 3–4 month. The rats were housed in sterile cages and kept at 25°Ċ with 12/12 light-dark cycle. The rats allowed for chow pellet and to drink tap water *ad libitum*. Humane care for animals was according to the conduct to the care and utilize of laboratory animal under ethical endorsement permission.

After 2 weeks of acclimatization period, the animals were randomly divided into three groups, 10 rats in each group.

- Group I: (Negative control group) Received normal saline (5 ml/kg/day) for 10 days.
- Group II: (Positive control group) Received normal saline (5 ml/kg/day) for 10 days, and on the 8th day, a single dose of doxorubicin (15 mg/kg, IP) (doxorubicin HCl 50 mg Pfizer USA)

soluble powder was given which serves as doxorubicin group.

- Group III: (Treated group) Received febuxostat (10 mg/kg, po), Feburic 120 mg (Alhikma co. Jordon) daily for 10 successive days, and on the 8th day, a 1 h after drug administration, a single dose of doxorubicin (15 mg/kg, I.P.) was given. The procedure of the study was according to Su *et al*. study [10].

At the 11th day of the study, rats were sacrificed and hearts were taken for histopathological observations, blood samples were taken for biochemical analysis. Blood samples were collected through intracardiac puncture in sterile labeled tubes, then centrifuged for 10 min at 4500 rpm and stored at −20°C to be assessed later.

Assessment of the biochemical variables

Serum brain natriuretic peptide (BNP), cardiac troponin I (cTn-I), caspase-3, glutathione peroxidase (GSH-Px), lipid peroxidase (LPO), malondialdehyde (MDA), and tumor necrosis factor alpha (TNF-α) were estimated by sandwich ELISA kit method (Kono Biotech Company, China).

Assessment of the histopathological changes

Fixation, tissue dissection, microtome sectioning, and slide preparation of rat hearts were according to Ashour *et al*. study [11].

Statistics

Data analysis was done using IBM SPSS (IBM SPSS Statistics for Windows version 20.0, 2014 Armonk, NY, IBM, Corp). The data were expressed as mean ± standard deviation. The significance of the difference of different means (quantitative data) was tested using Student's t-test for difference between two independent means. Statistical significance was considered when $p<0.05$.

RESULTS

Changes in the cardiac, inflammatory, oxidative, and apoptotic biomarkers in doxorubicin-induced acute cardiotoxicity

Results of the present study demonstrated that doxorubicin led to cardiotoxicity since it causes a significant elevation in the cardiac biomarkers. Cardiac troponin, BNP, caspase-3, and LPO were highly elevated compared to control $p<0.01$, while MDA and TNF-α were elevated but to a lesser extent $p<0.05$. In addition, GSH-Px serum levels were decreased significantly $p<0.01$ compared to control group, Table 1.

Febuxostat administration before doxorubicin led to a significant decrease in cardiac troponin, caspase-3, and elevation in GSH-Px levels significantly $p<0.05$. While the effect of febuxostat on BNP, LPO, MDA, tumor necrosis-alpha were insignificant $P>0.05$ compare to doxorubicin group Table 2.

Histopathological changes in doxorubicin-induced acute cardiotoxicity

The control group section showed normal structure of myocardial tissue with peripherally located normal oval nucleus and branching striated muscle fibers**.** While, the section of doxorubicin group showed many congested and dilated blood vessels with extravasations, edema, cytoplasmic vaculations, decreased number of nuclei, and loss of muscle fibers striation. Regarding the effect of febuxostat, it led to the preservation of nuclei without muscles fibers fragmentation, but congested and dilated blood vessels with edema and extravasations are seen, Fig. 1.

DISCUSSION

The present study clearly showed that doxorubicin induces myocardial injury by a significant elevation in plasma level of cTn-I in doxorubicin-treated rats compared to the control. These findings are in agreement with the experimental research that showed the response of CTn-I to chemotherapy-induced acute myocardial injury [12].

It is well-known that cardiac troponin is regarded as the gold standard biomarker for myocardial injury and cardiotoxicity. It is only released into the plasma when cardiac myocytes were injured, so an increase in its level during treatment with doxorubicin reflects the acute cardiotoxicity of this chemotherapeutic agent. Thus, an elevation of CTn-I after a high dose of chemotherapy accurately predicts the severity of cardiac dysfunction [13].

Table 1: Serum level of cardiac biomarkers during doxorubicin-induced cardiotoxicity in rats compared to the control

Parameters	Control (n=10)	Doxorubicin (n=10)	p
BNP (µg/L)	10.67±1.63	17.17+1.94	0.0001*
Caspase-3 (pmol/L)	13.33±2.42	24.67±4.59	0.0003*
Cardiac troponin (ng/L)	17±3.41	42.0±7.54	0.0001*
GSH (pmol/L)	24.83±3.97	14.5±4.32	0.001*
LPO (nmol/L)	14.83±1.72	26.17±7.83	0.006*
MDA (nmol/L)	1.1±0.414	1.933±0.74	0.03
TNF (ng/L)	23.17±11.09	37.33±8.96	0.03

Results are expressed as mean±SD; *p<0.01, p<0.05, BNP: Brain natriuretic peptide, GSH: Glutathione peroxidase, LPO: Lipid peroxidase, MDA: Malondialdehyde, TNF: Tumor necrosis factor alpha

Table 2: Effects of febuxostat on cardiac biomarker levels during doxorubicin-induced cardiotoxicity in rats

Parameters	Doxorubicin (n=10)	Febuxostat (n=10)	p
BNP (µg/L)	17.17+1.94	15.8±1.788	0.25
Caspase-3 (pmol/L)	24.67±4.59	20.4±1.14	0.03*
Cardiac troponin (ng/L)	42.0±7.54	32.8±4.21	0.03*
GSH (pmol/L)	14.5±4.32	19.2±3.34	0.02*
LPO (nmol/L)	26.17±7.83	24.6±2.23	0.40
MDA (nmol/L)	1.933±0.74	1.60±0.15	0.14
TNF (ng/L)	37.333±8.96	34.0±3.83	0.25

Results are expressed as mean±SD; *p<0.05, BNP: Brain natriuretic peptide, GSH: Glutathione peroxidase, LPO: Lipid peroxidase, MDA: Malondialdehyde, TNF: Tumor necrosis factor alpha

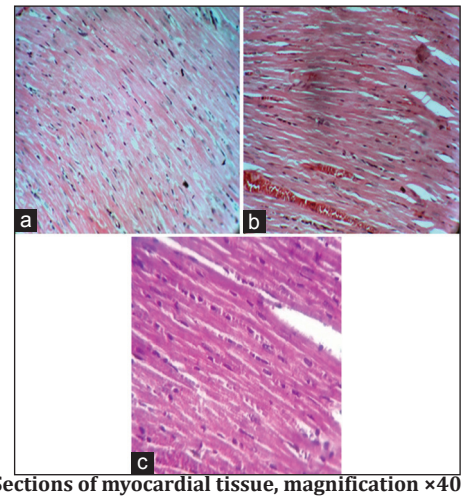

Fig. 1: Sections of myocardial tissue, magnification ×40 (H and E). (a) (Control) Section showed normal rat myocardial tissue, (b) (doxorubicin) section showed congested and dilated blood vessel fragmented muscles fibers (orange arrow), decreased number of nuclei due to doxorubicin-induced acute cardiotoxicity, (c) (febuxostat) section showed preserved nuclei and no muscles fibers fragmentation but congested and dilated blood vessels with edema and extravasations due cardioprotective effect of febuxostat

Therefore, the results of the present study pointed out that rats which were pre-treated with febuxostat showed reduction in CTn-I which is agreed with the previous study that has been described the response of cardiac troponins to DIC [14].

As well, the present study showed a significant increase in LPO in doxorubicin group compared to control group that corresponds with Khan *et al*. study that demonstrated a significant elevation in LPO levels in DIC [15]. Increase in LPO level could be attributed to the cardiomyocyte toxic effects of free radical oxidative degradation of doxorubicin [16].

Moreover, the results of the present study illustrated that rats which pre-treated with febuxostat showed significant decrease in LPO serum level compared to doxorubicin-treated rats, this finding is in agree with Wang *et al.* study that showed febuxostat therapy in animal model study causes a significant decline in serum levels of LPO due to inhibition of free radical generation [17].

Furthermore, MDA levels were significantly increased in the doxorubicin-treated rats compared to control group. This finding confirms the role of free radicals in DIC. Increase in MDA level could be attributed to the effects of free radicals that are generated as a result of doxorubicin effect, on NADH-dependent microsomal LPO that initiates lipid radical chain and oxidative damage [18,19]. Also, pre-treatment with febuxostat led to significant decrease in MDA serum level compared to doxorubicin treated rats which coincided with a study that demonstrated a significant decrease in MDA serum levels during febuxostat treatment [20].

Furthermore, TNF was significantly increased in doxorubicin group compared to the control group. Interestingly, both myocardial macrophages and cardiac myocytes synthesize TNF-α. Accumulating evidence indicates that myocardial TNF-α is an autocrine contributor to myocardial dysfunction and cardiomyocyte death in ischemia-reperfusion injury a sepsis and chronic heart failure [21].

Moreover, pre-treated with febuxostat showed insignificant decrease in serum TNF-α level compared to doxorubicin treated rats. This outcome is not corresponded with a study that disclosed a potential effect of febuxostat in reduction of pro-inflammatory cytokines [22].

In the present study, BNP was significantly increased in the doxorubicin-treated rats compared to control group. In contrast, Ruggiero *et al.* reported low BNP serum levels during acute DIC due to inhibition of BNP gene expression [23]. However, in the present study, high levels of BNP were due to the development of acute heart failure as BNP serum levels are correlated with the severity of heart failure [24].

The cardioprotective effects of febuxostat might explain the reduction in BNP serum levels as febuxostat illustrates antioxidant and anti-inflammatory effects as well as the reduction of serum uric acid which have deleterious effect on the cardiomyocyte [17].

Moreover, the present study showed that febuxostat decreases caspase-3 serum level compared to doxorubicin-treated rats. This results in agreement with the research that showed febuxostat reduced the apoptotic marker, namely caspase-3 compared to doxorubicin-treated rats due to the antiapoptotic effect of febuxostat [25].

Regarding the effect on the endogenous antioxidant capacity, doxorubicin produced significant decreases of GSH-Px in DIC compared to the control group due to free radicals formation that attacks the glutathione. Febuxostat significantly improves body endogenous capacity through augmentation of antioxidant enzymes. On the other hand, febuxostat was ineffective in reduction of MDA serum levels and oxidative stress which might due to small dose of febuxostat in neutralization the effect of free radicals [26].

In the present study, structural changes that demonstrated microscopically in the heart of doxorubicin group compared to normal control group. Doxorubicin induced cardiac cell injury which revealed as congested and dilated blood vessels, extravasations, edema, cytoplasmic vaculations, decreased number of nuclei, loss of muscle fibers striation and fragmentation with necrosis [27].

In rats treated with febuxostat, the cardiac cells maintained their integrity by the evidence of preserved nuclei, the absence of fragmentation of the muscle fibers. It has been reported that febuxostat attenuated the cardiac cell injury by its antioxidant effect. Febuxostat limits the infarct size of acute myocardial infarction due to its antioxidant, anti-inflammatory, and antiapoptotic effect [28].

CONCLUSION

Febuxostat attenuates DIC through modulation of antioxidant, anti-inflammatory, and antiapoptotic pathways.

AUTHORS' CONTRIBUTIONS

All authors contribute equally in data collection, experimental design, interpretation, statistical analysis, literature review, manuscript preparation, and review.

CONFLICTS OF INTEREST

Nil.

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