

## Ginkgo Biloba protects cardiomyocytes against acute doxorubicin induced cardiotoxicity by suppressing oxidative stress

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### Abstract

**Objectives:** To evaluate the cardio-protective effect of *Ginkgo Biloba* (GB) on doxorubicin induced-cardiotoxicity.

**Methods:** The experimental study was conducted at the College of Medicine, Mustansiriyah University, Baghdad, Iraq, from January to March, 2016, and comprised thirty Wistar Sprague male rats aged 3-4 months and weighing 200-400 g. The rats were divided into three equal groups (n=10); Group I (control): rats were treated with distilled water, Group II (doxorubicin): rats were treated with distilled water and doxorubicin 20 mg/kg, and Group III (GB): rats were treated with GB and doxorubicin 20mg/kg. Serum malondialdehyde (MDA), glutathione reductase (GSH), lipid peroxidase (LPO), tumour necrosis factor-alpha (TNF- $\alpha$ ), cardiac troponin (cTnI), brain natriuretic peptide (BNP) and caspase-3 (Cas-3) were measured using enzyme-linked immunosorbent assay kits. SPSS 20 was used to compare the effect GB with doxorubicin on the biomarkers of doxorubicin induced-cardiotoxicity.

**Results:** Doxorubicin led to cardiotoxicity through elevation of cTnI, BNP, Cas-3 and LPO compared with controls (p<0.01). Also, MDA and TNF- $\alpha$  were elevated while; GSH was decreased significantly (p<0.01) compared with controls. Co-administration of GB with doxorubicin led to significant reduction in cTnI, Cas-3 sera levels with elevation in GSH serum level significantly (p<0.05). The effect of GB on BNP, LPO, MDA and TNF- $\alpha$  was insignificant (p>0.05) compared with the doxorubicin.

**Conclusion:** GB has significant cardio-protective effect through attenuation of oxidative stress during doxorubicin induced-cardiotoxicity in rats.

**Keywords:** Doxorubicin, Ginkgo Biloba, Cardiotoxicity. (JPMA 69: S-103 (Suppl. 3); 2019)

### Introduction

Doxorubicin is a chemotherapeutic drug used in combination with other anti-cancer drugs for the treatment of sarcomas and carcinomas as well as acute lymphocytic leukaemia. Doxorubicin induced-cardiotoxicity (DIC) is a cumulative dose-dependent progressive myocardial damage which leads to heart failure.<sup>1</sup>

Doxorubicin leads to apoptosis, necrosis and autophagy through formation of free radicals. The heart is more vulnerable to the effect of free radicals due to high density of mitochondria which is the main site for the creation of free radical.<sup>2</sup> During DIC, mitochondrial calcium-loading capacity is lost which results in the induction of mitochondrial permeability transition pore (MPTP), which increases the inner mitochondrial membrane permeability.<sup>3</sup> Additionally, doxorubicin inhibits endothelial nitric oxide synthase (eNOS), result in

reduction of nitric oxide (NO) production. This interaction is dose-dependent and high dose of doxorubicin deviate eNOS for the generation of superoxide instead of NO. Genetically absence of eNOS transcription protects against the DIC.<sup>4</sup> Indeed, in DIC there is over-expression of both  $\beta$ -adrenergic receptors and pro-apoptotic pathway leading to heart failure.<sup>5</sup> As well, doxorubicin may cause important dys-regulation of cardiomyocyte calcium content by direct or indirect effect through the induction of reactive oxygen species (ROS). Dys-regulation of cardiomyocyte calcium occurs through inhibition of sarcoplasmic reticulum calcium pump, sarcolemma of sodium/potassium pump and ryanodine receptor.<sup>6</sup>

The clinical presentation of DIC including; acute cardiotoxicity, early onset-cardiotoxicity and late onset-cardiotoxicity.<sup>7</sup>

Therefore, uses of natural or synthetic anti-oxidants may protect against DIC through modulation of oxidative stress and preservation of endogenous antioxidant capacity.<sup>8</sup>

Ginkgo Biloba (GB) is a herbal agent belonging to the Ginkgoaceae family. The main constituents of GB extracts are flavonoids, terpenoids and various organic acids.

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Flavonoids are responsible for the major anti-oxidant effect of GB, which reduced the level of free radicals and prevent lipid peroxidation.<sup>9</sup>

Therefore, the aim of the present study was to evaluate the cardio-protective effect of Ginkgo Biloba (GB) on doxorubicin induced-cardiotoxicity in rats.

## Materials and Methods

This experimental study was conducted in the Department of Pharmacology, College of Medicine, Mustansiriyah University, Baghdad, Iraq, from January to March, 2016, and comprised thirty Wistar Sprague male rats aged 3-4 months and weighing 200-400 grams each. The animals were obtained from the International Centre of Cancer and Medical Researches, College of Medicine, Mustansiriyah University Baghdad-Iraq. The rats were placed in cages at suitable room temperature and artificial 12/12hrs light-dark cycle. They left for one week for acclimatisation without any intervention and with free access to normal chow pellets and water ad libitum. Care for animals was taken according to the Guide to the Care and Use of Laboratory,<sup>10</sup> animals after getting approval from the institutional ethics committee.

After the acclimatisation period, the rats were randomly divided into three equal groups (n=10). The induction of cardiotoxicity was done in line with literature.<sup>11</sup>

Group I (control): rats were treated with distilled water 5 ml/kg/day orally for 10 days. Group II (doxorubicin): rats were treated with distilled water 5 ml/kg orally for 10 days and on 8th day they received single intra-peritoneal injection of doxorubicin 20 mg/kg. Group III (GB): rats were treated with GB 100 mg/kg/day for 10 days and on 8th day they received single intra-peritoneal injection of doxorubicin 20 mg/kg.

On 11th day, rat decapitation was done under chloroform anaesthesia, and 3-4ml of blood samples was centrifugated for 10 minutes at 5000 rpm at room temperature. The formed sera were isolated and kept at -20°C till the time of analysis.

## Biochemical Variables

Serum malondialdehyde (MDA), glutathione reductase (GSH), lipid peroxidase (LPO), tumour necrosis factor-alpha (TNF- $\alpha$ ), cardiac troponin (cTnI), brain natriuretic peptide (BNP) and caspase-3(Cas-3) were measured by using enzyme-linked immunosorbent assay (ELISA) kit method as per the instructions given by the manufacturers (Myo-bio source, USA).

## Histopathological Variables

For tissue sample collection, the heart was separated from the decapitated animals, and stored in normal saline solution. The isolated hearts were fixed in 10% formalin buffer to preserve the tissue structure according to the paraffin methods.<sup>12</sup> The staining of the tissue sections was done by using haematoxylin and eosin (H&E) stains.

## Statistical Analysis

Data analysis was done using SPSS 20, and it was presented as mean $\pm$  standard deviation (SD). The variables were tested using unpaired student t-test to compare treated groups with control. The levels of significance was set at p<0.05.

## Results

Doxorubicin led to cardiotoxicity through elevation of cTnI, BNP, Cas-3 and LPO compared with controls (p<0.01). Also, MDA and TNF- $\alpha$  were elevated while; GSH was decreased significantly (p<0.01) compared with controls (Table-1). Co-administration of GB with doxorubicin led to

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

Parameters	Control (n=10)	Doxorubicin (n=10)	% changes	P
BNP ( $\mu$ g/L)	10.67 $\pm$ 1.63	17.17 $\pm$ 1.94	+60.94	0.0001*
Caspase-3 (pmol/L)	13.33 $\pm$ 2.42	24.67 $\pm$ 4.59	+84.9	0.0003*
cTn (ng/L)	17 $\pm$ 3.41	42.0 $\pm$ 7.54	+147	0.0001*
GSH (pmol/L)	24.83 $\pm$ 3.97	14.5 $\pm$ 4.32	-41.6	0.001*
LPO (nmol/L)	14.83 $\pm$ 1.72	26.17 $\pm$ 7.83	+76.4	0.0061*
MDA (nmol/L)	1.1 $\pm$ 0.414	1.933 $\pm$ 0.74	+75.7	0.03**
TNF- $\alpha$ (ng/L)	23.17 $\pm$ 11.09	37.333 $\pm$ 8.96	+61.1	0.03**

Results are expressed as mean $\pm$  SD; \*p<0.01, \*\*p<0.05, BNP: Brain natriuretic peptide, GSH: Glutathioneperoxidase; cTn: Cardiac troponin; LPO: Lipid peroxidase; MDA: Malondialdehyde; TNF: Tumour necrosis factor alpha.

Table-2: effects of Ginkgo Biloba on cardiac biomarker levels during doxorubicin-induced cardiotoxicity in rats compared to the controls.

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

Results are expressed as mean $\pm$  SD; \*p<0.01, p<0.05, BNP: Brain natriuretic peptide, GSH: Glutathioneperoxidase; cTn: cardiac troponin; LPO: Lipid peroxidase; MDA: Malondialdehyde; TNF: Tumour necrosis factor alpha.

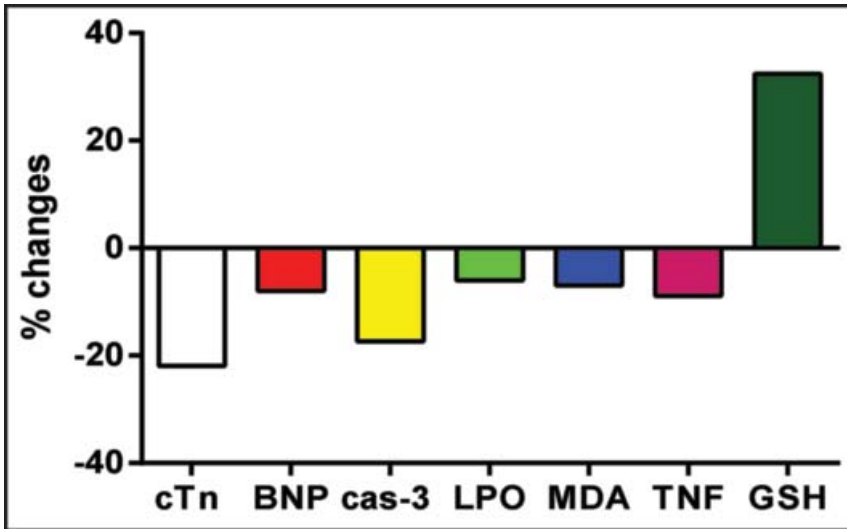


Figure-1: Changes in cardiac biomarkers due to administration of Gingko Biloba during doxorubicin-induced cardiotoxicity.

significant reduction in cTnI, Cas-3 sera levels with elevation in GSH serum level significantly ( $p < 0.05$ ). The effect of GB on BNP, LPO, MDA and TNF- $\alpha$  was insignificant ( $p > 0.05$ ) compared with the doxorubicin (Table-2). Percent changes illustrated a decline in cTnI, BNP, Cas-3, LPO, MDA and TNF- $\alpha$  with significant elevation of GSH (Figure-1).

Regarding histopathological changes, GB reduced myocardial damage, muscles fibre fragmentation with preservation of nuclei. However, congested and dilated blood vessels were still present with less frequent inflammatory and apoptotic cells (Figure-2).

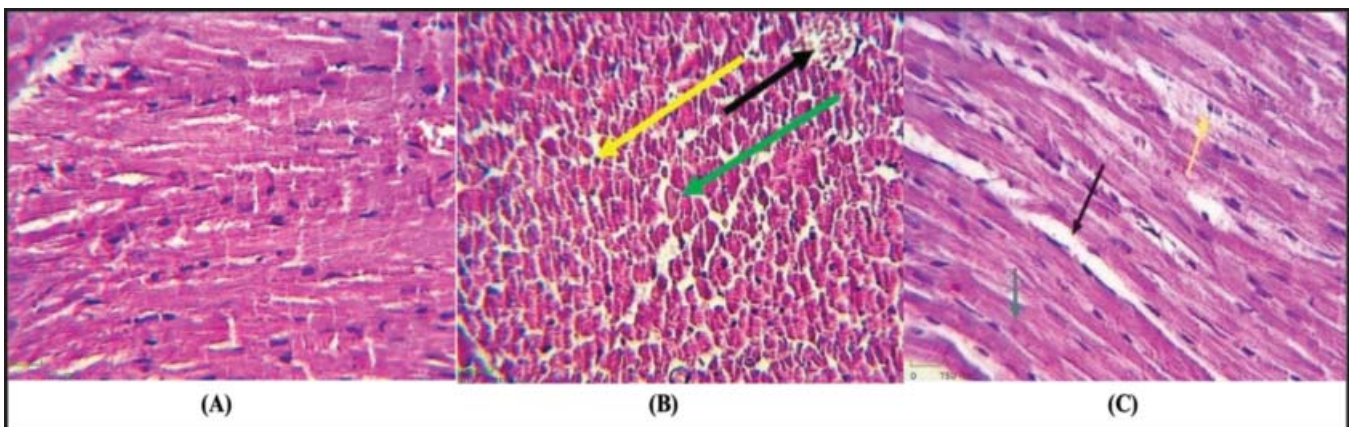


Figure-2: Histopathological sections of myocardial tissue. (A): Shows normal myocardial tissue, magnification 40X, hematoxylin and eosin (H&E). (B): Showed myocardial tissue, congested and dilated blood vessel (black arrow) with inflammatory cells (yellow arrow) and apoptotic cell (green arrow), magnification 40X, (H&E). (C): Showed improved myocardial damage, preserved nuclei and less muscles fibre fragmentation but congested and dilated blood vessels are still present (black arrow), less frequent inflammatory cells (yellow arrow) and less apoptotic cells (green arrow), 40X, (H&E).

**Discussion**

In the present study, DIC was established by an elevation in the serum level of BNP compared with controls which was in agreement with several studies that described the response of BNP in DIC.<sup>13</sup> The elevation in the serum levels of BNP was due to the development of cardiomyopathy and left ventricular dysfunction (LVD). When the heart is injured, BNP is rapidly secreted from the heart tissue, as showed in a previous study.<sup>14</sup> Therefore, BNP elevation in the present study might be due to the occurrence of acute heart failure by high doxorubicin dose.<sup>15</sup>

In the present study, pre-treatment with GB had no significant effect on serum BNP level which was in contrast with Khafaga et al study that showed significant reduction in BNP serum levels upon GB administration.<sup>16</sup> However; our result might be due to short duration of the study or small dose of GB to counter the full prone effect of doxorubicin.

The present study pointed out that doxorubicin causes significant elevation in serum levels of Cas-3 in DIC compared with controls, which corresponded with Wang et al study that found doxorubicin elevates Cas-3 level and activate apoptosis by decreasing the expression of anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein and augmented oxidative stress by increasing oxygen species production.<sup>17</sup>

Moreover, the present study illustrated that pre-treatment with GB had a significant effect on the reduction of Cas-3 level in DIC through suppression of caspase-3/9 activity due to its anti-oxidant, anti-inflammatory and anti-apoptotic effects of GB.<sup>18</sup>

Besides, the present study illustrated that doxorubicin caused significant decrease in glutathione peroxidase (GPx) serum level in DIC as noted by a previous study that illustrated doxorubicin causes significant reduction in GPx serum level due to the production of free radicals.<sup>19</sup> Therefore, pre-treatment with GB led to a significant elevation in GPx serum in DIC as supported by Henriksen study that confirmed the anti-oxidant ability of GB.<sup>20</sup>

Indeed, the present study illustrated that cardiac injury induced by doxorubicin led to an increase in cTnl level which agrees with earlier studies.<sup>21</sup> Also, the present study illustrated that GB had a significant effect in the reduction of cTnl serum level due to important cardio-protective effect as revealed by Da Rocha et al.<sup>22</sup>

Additionally, our finding showed that lipid peroxidation was elevated in doxorubicin-treated group compared with control due to mitochondrial dysfunction, iron-dependent oxidative damage, production of reactive oxygen species, and depletion of endogenous antioxidants.<sup>23</sup>

A recent study showed that GB has ability in lowering lipid peroxidation due to its anti-oxidant effect.<sup>24</sup> But in the present study; GB had no significant effect on lipid peroxidation which might be due to short duration of the study and/or low dose of GB.

It has been reported that elevation of MDA level is correlated with the increment of lipid peroxidation in DIC due to transformation of doxorubicin into semiquinone radical that reduces oxygen and form superoxide which reacts with polyunsaturated fatty acids to generate lipid hydroperoxide and MDA as the final product of lipid peroxidation process.<sup>25</sup> The present study demonstrated that GB had no significant effect on MDA serum level compared with control, but an earlier study showed a significant reduction in MDA level.<sup>26</sup>

Indeed, the present study showed an increase in TNF- $\alpha$  in DIC compared with control as supported by Tian et al study that revealed an elevation in TNF- $\alpha$  serum level in doxorubicin cardiomyopathy may be due to the inflammatory changes.<sup>27</sup>

However, in the present study, pre-treatment with GB produced no significant effect on serum TNF- $\alpha$  which might be due to the difference in combination

percentage of GB extracts (flavonoids and terpenoids). In addition, results of the present study showed significant histo-pathological changes on the cardiac cells due to the cardio-protective effect of GB that reduces cardiomyocyte injury and preserves histological architectures.<sup>28</sup>

## Conclusion

Ginkgo Biloba was found to have a significant cardio-protective effect through attenuation of oxidative stress during doxorubicin-induced cardiotoxicity in rats.

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**Conflicts of Interest:** None.

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