Pharmacological study of potential effect of chrysin in doxorubicin-induced cadiotoxicity in rats

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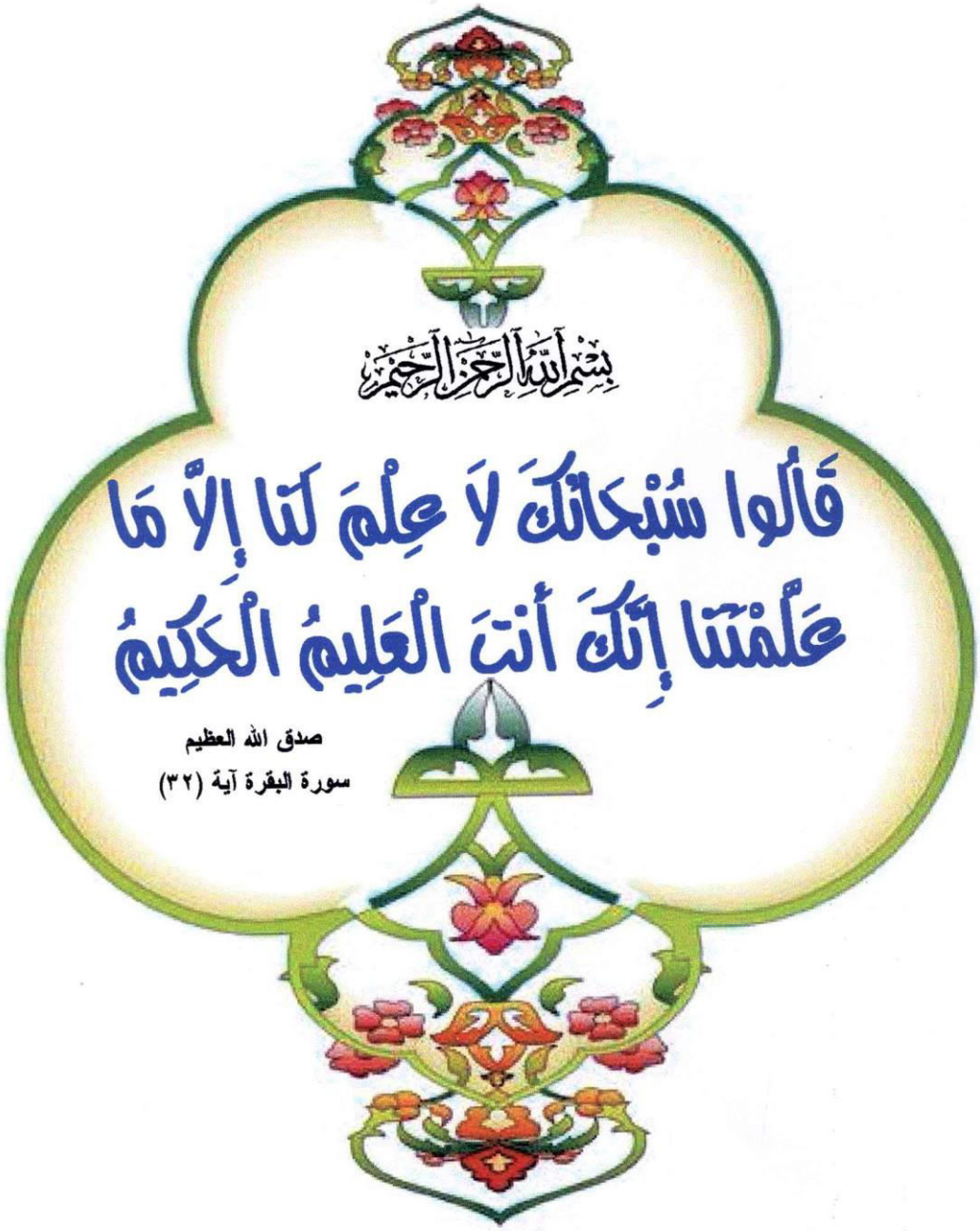
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Doxorubicin (DOX) is one of the most effective chemotherapeutic drugs; however, its incidence of cardiotoxicity compromises its therapeutic index. Chrysin, a natural flavone, possesses multiple biological activities, such as antioxidant, anti-inflammatory and anti-cancer. The current study was divided into two parts. The first part aimed to screen the cardioprotective dose of chrysin where male albino rats received chrysin once daily for 12 consecutive days at doses of 25 and 50 mg/kg orally followed by a single dose of DOX (15 mg/kg; i.p.) on day 12. DOX induced significant myocardial damage in rats, which was characterized by conduction abnormalities, decreased heart-to-body weight ratio, increased serum CK-MB and LDH and myofibrillar disarrangement. These effects were almost prevented by pretreatment with chrysin at dose of 50 mg/kg which was further used in the second part of the study that aimed to elucidate the possible underlying molecular mechanisms of the potential cardioprotective effect of chrysin. Male albino rats were treated with either DOX (5 mg/kg, i.p., once a week) and/or chrysin (50 mg/kg, orally, four times a week) for four weeks. As indicators of oxidative stress, DOX caused significant GSH depletion, lipid peroxidation and reduction in activities of antioxidant enzymes; CAT, SOD, Gpx and GR. Co-treatment with chrysin significantly attenuated DOX-induced oxidative injury in cardiac tissue. Furthermore, DOX induced apoptotic tissue damage by increasing the expression of p53, Bax, Puma and Noxa and caspase-3

activity while decreasing the expression of Bcl-2. Chrysin co-treatment ameliorated these apoptotic actions of DOX. Moreover, DOX induced activation of MAPK; p38 and JNK and increased the expression of NF-κB which further promote the DOX-induced apoptotic cell death. Meanwhile, DOX decreased the activation of AKT survival pathway via increasing the gene expression of its inhibitory enzyme; PTEN. On the contrary, chrysin co-treatment effectively neutralised all these effects. Collectively, these findings indicate that chrysin possesses a potent protective effect against DOX-induced cardiotoxicity via suppressing oxidative stress and apoptotic tissue damage.

Keywords: Cardiotoxicity; Doxorubicin; Chrysin; Oxidative stress; Apoptosis

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List of Abbreviations

4-AAP	4- Aminoantipyrine.
AIF	Apoptosis inducing factor
Apaf-1	Apoptosis protease activation factor-1
ATP	Adenosine triphosphate
BAD	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist/killer
Bax	Bcl-2 associated X protein
BCA	Bicinchoninic acid
Bcl-2	B-cell lymphoma 2
Bcl-xl	B-cell lymphoma-extra large
BDZ	Benzodiazepines
BID	BH3 interacting-domain death agonist
BSA	Bovine serum albumin
CAT	Catalase
CD4	Cluster of differentiation 4
C/EBP δ	CCAAT/enhancer binding protein δ
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary DNA

cGMP	Cyclic guanosine 3′,5′- monophosphate
CHF	Congestive geart failure
CK-MB	Creatine kinase MB
COX-2	Cyclooxygenase- 2
Ct	Cycle threshold
DCHBS	3,5-dichloro -2-hydroxybenzene sulfonic acid 4-aminophenazone 4-AAP
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOX	Doxorubicin
dsDNA	Double stranded DNA
DSS	Dextrane sodium sulfate
DTNB	5,5'-dithio-bis (2-nitrobenzoic acid)
DTT	Dithiothreitol
DW	Distilled water
ECG	Electrocardiography
ECL	Enhanced luminol based chemiluminescent
EDTA	Ethylene-diamine tetraacytic acid
EndoG	Endonuclease G
ERK	Extracellular signal-regulated kinase

FLIP	FLICE/caspase-8 inhibitory protein
Fp	Flavoprotein;
G_6PDH	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HK	Hexokinase
HRP	Horseradish peroxidase
IFN-γ	Interferon-γ
IGF-1	Insulin-like growth factor-1
I κ B	Inhibitor of kappa B
IKK	IκB kinase
IL	Interlukin
iNOS	Inducible nitric oxide synthase
IP	Intrapeitoneal
IV	Intravenous

JNK	c-Jun N-terminal kinase
LDH	Lactate dehydrogenase
LPS	Liopopolysaccahride
MAPK	Mitogen-activated protein kinase
Mcl-1	Myeloid leukemia cell differentiation protein
MDA	Malondialdehyde
mRNA	Messenger RNA
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
O^{2-}	Superoxide anion
PG	Prostaglandin
PI3K	Phosphoinositide kinase
PMS	Phenazine methosulphate
pNA	p-nitroaniline
PTEN	Phosphatase and tensin homolog

Puma	P53 upregulated modulator of apoptosis
PVDF	Polyvinyl difluoride
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RQ	Relative quantitation
RT-PCR	Real time polymerase chain reaction
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBS	Tris-buffered saline
TBST	Tris-buffered saline with tween
TCA	Trichloroacetic acid
TEMED	Tetramethylethylenediamine
Th	T-helper type
TNF-α	Tumor necrosis factor-alpha
TRAIL	TNF-related apoptosis-inducing ligand

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