Zagazig University Medical Journal
www.zumj.journals.ekb.eg
Clinical Pharmacology

ORIGINAL ARTICE

Hypericum perforatum alleviates doxorubicin induced cardiotoxicity in rats via enhancement of cardiac adiponectin-AMPK signaling

Nisreen E. Elwany*, Nevertyty Mohamed Mahmoud, Amira Mohamed Abdel Hamid
Clinical Pharmacology Department, Faculty of Medicine, Zagazig University, Egypt.

***Corresponding author:**
Dr :Nisreen E. Elwany

Clinical Pharmacology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Email:
NAAlwan@medicine.zu.edu.eg

ABSTRACT

***Background*:** Doxorubicin (DOX) is commonly used for treatment of hematologic and solid malignancies but its dose related cardiotoxicity limits its use. Therefore, this study aims to evaluate the possible protective effect of Hypericum perforatum (HP) against doxorubicin cardiotoxicity.

***Methods*:** Rats were allocated into 4 groups: **GI**: received normal saline *i.p.*; **GII**: received DOX (15 mg/kg; single *i.p.* injection); **GIII**: received HP 125 mg/kg/day *p.o* for 3 weeks + DOX; **GIV**: received HP 250 mg/kg/day *p.o.* for 3 weeks+ DOX.

ECG was performed then the animals were sacrificed. Sera were collected for estimation of LDH, CK-MB and cTnT. Hearts were isolated for histopathological examination and determination of APN, adiponectin receptor 1, adiponectin receptor 2, AMPKα1, AMPKα2, GSH, and MDA. SOD and catalase activities besides, the gene expression of BCL2, Bax and caspase 3 were also determined.

***Results*:** Dox induced bradycardia, widening of QRS complex and prolongation of PR intervals, increased serum LDH, CK-MB and cTnT, and promoted cardiomyocyte inflammation and apoptosis. Additionally, Dox increased Bax and caspase 3, while decreasing BCL2 gene expression. Meanwhile, cardiac GSH, SOD, CAT, and GPx were decreased along with elevation of MDA. Furthermore, Dox decreased cardiac Adiponectin (APN), adiponectin receptor 1, adiponectin receptor 2, AMPKα1 and AMPKα2 gene expression. On the other hand, pretreatment with HP improved all the aforementioned parameters in a dose dependent fashion, with amelioration of cardiomyocyte inflammation and apoptosis compared with DOX group.

Conclusions: HP protects against DOX cardiotoxicity via enhancement of cardiac adiponectin-AMPK signaling.

Key words: Adiponectin; Apoptosis; Cardiotoxicity; Doxorubicin; Hypericum perforatum.

Abbreviation: Hypericum perforatum (HP); Doxorubicin (DOX), Intraperitoneal (i.p.); Lactate dehydrogenase (LDH); Creatine kinase-MB (CK-MB); Cardiac troponin T (cTnT); Reduced glutathione (GSH); Malondialdhyde (MDA); Superoxide dismutase (SOD); Catalase (CAT) activities ; Adiponectin (APN). St. John's wort.

INTRODUCTION

Doxorubicin (DOX) is an anthracycline chemotherapeutic drug that is commonly used for treatment of hematologic and solid malignancies **[1]**. In spite of the effectiveness of DOX in treating cancer, its therapeutic use is limited due to its associated cardiotoxicity **[2].** Therefore, preventive measures are needed to mitigate DOX- induced cardiotoxicity and to enhance its safety in cancer treatment. One of the mechanisms by which DOX induces cardiomyocyte injury is the excessive generation of intracellular reactive oxygen species

(ROS) that overwhelm myocardium antioxidant capacity. Additionally, DOX can promote myocardial apoptosis [**3, 4].**

On the other hand, adiponectin (APN) is an adipokine released by adipocytes with its anti-inflammatory, antioxidant, and anti-fibrotic properties **[5]**. APN expression was identified in the myocardium. and it was reported to have cardiovascular protective effects **[6, 7, 8].** In addition to APN ability to diminish oxidative stress, it can attenuate DOX- induced apoptosis and increase the cell survival **[9, 10, 11].** Konishi et al. **[12]** found that the APN mitigated the DOX-induced myocardial fibrosis and apoptosis via modulation of adenosine monophosphate-activated protein kinase (AMPK) signaling.

Hypericum perforatum (HP), also known as St. John's wort, is a herbal medication with strong antidepressant properties. HP was evidenced to be effective in alleviating anxiety and moderate depression **[13].** It has been demonstrated that HP has antioxidant properties [**14].** In addition, it has been suggested to possess analgesic, anti-inflammatory, antimicrobial and antiviral activities [**15].** Many studies showed that HP could be useful to treat obesity, insulin resistance **[16, 17],** periodontitis **[18],** nephrotoxicity **[19]** and diabetic nephropathy **[20].** Researchers showed that HP increased the expression of adiponectin (APN) in adipocytes. The HP infusion enhanced APN levels in obese rats [**21].** Yet, it is not evidenced if HP can modulate APN expression in the cardiomyocyte. Therefore, aim of the current investigation is to assess any potential protective effects of HP in the rat model of DOX-associated cardiotoxicity through modulating the cardiac adiponectin-AMPK signaling.

Materials and Methods

Animals

Twenty four adult male *Wistar albino* rats weighing 200– 250gm were utilized in the present investigation. They were brought from Faculty of Veterinary Medicine, Zagazig University, Egypt. The rats were kept in a room temperature (22 ± 2C) and humidity (48% relative humidity). The animals were fed commercial pelleted rat chow and tap water ad libitum. The study was approved by the institutional research board of the faculty of Medicine, Zagazig University, which follows the US National Institutes of Health guidelines for animal care.

Experimental design and dosing protocol

After acclimatization, the animals were randomly divided into four groups consisting of 6 rats each: **GI**: normal control group: the rats received a single dose of isotonic saline by i.p. injection. **GII:** DOX treated rats: the rats received a single dose of doxorubicin 15 mg/kg dissolved in normal saline i.p. **[22]**. **GIII**: (HP 125+DOX): HP pretreatment at a dose of 125 mg/kg/day; p.o. for 3 weeks + DOX (15 mg/kg; a single i.p. injection). **GIV**: (HP 250+DOX): HP pretreatment at a dose of 250 mg/kg/day; p.o.; for 3 weeks + DOX (15 mg/kg; a single i.p. injection)**.** Doses of HP (ATOS Pharma, ARE) were chosen based on previous study **[23].** HP was given for 3 weeks then DOX (Kyowa Hakko Kogyo Co. Ltd .Tokyo, Japan) was given. HP was then continued for another 3 days. At the end of experimental period, ECG was recorded; sera were collected for determination of total lactate dehydrogenase (LDH), creatine kinase- myocardial fraction (CK-MB) and cardiac Troponin T (cTnT). After scarification, the hearts were immediately separated to prepare specimens from the cardiac walls. One half of the specimens from all groups were fixed immediately in a10% neutral-buffered formalin solution and processed for histopathological examination. The second half was kept at -80˚C for myocardial tissue parameters estimations.

E.C.G. recording

The animals were put in a supine position on heated pads after being anesthetized with urethane (1.2 g/kg). Subcutaneous electrodes were placed into the left hind limb, left forelimb, and right forelimb in that order to record ECGs. Meanwhile, the needle electrode was placed into the right forelimb to records the lead II ECG [**24].**

Biochemical parameters

LDH activity was measured colorimetrically by kits obtained from Sigma-Aldrich. cTnT was determined using Elecsys, electrochemiluminiscence (ECLIA) sandwich immunoassay (Roche Diagnostics). Additionally, serum CK-MB was estimated by the Elecsys CK-MB (Roche Diagnostics).

Histopathological Examination.

The heart tissue was fixed in formalin10% for 24 hours, and then embedded in paraffin. After that, 5 um-thick slices were stained with hematoxylin and eosin for light microscope examination **[25]**.

Myocardial tissue parameters

The rat hearts were homogenized in 100 mM tris–HCl (pH 7.4), and then were centrifuged at 10,000 × g at 4 ◦C to separate the homogenate. The supernatant was used for evaluation of reduced glutathione (GSH), superoxide dismutase (SOD) activity, catalase activity (CAT), glutathione peroxidase (Gpx) and malondialdhyde (MDA) levels, while the residue was used for the analysis of adiponectin (APN), adiponectin receptor 1 , adiponectin receptor 2 , AMPKα1, AMPKα2, BCL2, Bax, caspase 3 gene expression.

Determination of oxidant/anti-oxidant status:

Myocardial GSH, SOD, CAT, Gpx and MDA were determined by colorimetric assay kits (Biodiagnostic, Cairo, Egypt), according to the manufacturer's guidelines.

Gene expression analysis

RNA extraction and quantitative real- time PCR

RNA extraction kits (RNeasy FFPE Kit, Qiagen) were used for extraction of total RNA from myocardial tissue homogenate. cDNA was synthesized from mRNA using reverse transcription kits (Reverse Transcriptase Master Mix, Roche Diagnostics) following the manufacturer's protocol. The expression of APN, adiponectin receptor 1, adiponectin receptor 2, AMPK, BCL2, Bax, and caspase 3 were detected by real time-PCR. The expressed data were normalized to Glyceraldehyde-3- phosphate dehydrogenase (GAPDH) gene.

Quantitative real-time PCR assay was performed in a mixture of 5 µL cDNA, 10 µL Eva Green-Mix (Jena Bioscience) and 0.6 µL primers (10 µM), PCR grade water up to 20 µL. Real time PCR (StratageneMx3005P-qPCR System) was used for amplification. The thermal cycling conditions for the genes were: initial denaturation and polymerase activation at 95 °C for 30 s, then 40 cycles of denaturation 95 °C for 15 s; annealing and elongation at 58 °C for 1 min. $2^{-\Delta\Delta CT}$ method was used for calculation of the relative changes in gene expression. The primers' sequences were shown in Table 1.

Statistical analysis

All the results were represented as means ± SD. Statistical significance among groups was estimated by one-way analysis of variance (ANOVA) followed by Post-Hoc (least significant difference "LSD") tests. When $P < 0.05$, results were considered statistically significant. The statistical analysis was carried out using Statistical package of social sciences (SPSS) computer software (version 16).

Results

ECG changes

Several ECG changes were observed in rats in DOX-treated group such as: a significant slowing of heart rate (32%), widening of QRS complex (93%) and prolongation of PR intervals (59%), as compared to rats in the control group. Those ECG alterations were ameliorated by HP pretreatment at both doses 125 and 250 mg/kg as evidenced by significant increase of the heart rate (23.5, 39%), significant reduction of QRS complex (18.3, 35.5%) and significant reduction of PR intervals (15.3, 27.6%) respectively, as compared to the DOX group (Figure 1& Table2).

Cardiotoxicity indices: Effect on serum LDH, CK-MB and cTnT.

Table 3 shows that DOX provoked a significant ($p<0.05$) increase in the serum values of LDH, CK-MB and cTnT by (322, 198, 690 %) respectively, when compared to the control group. Treatment with HP at 125, 250 mg/kg dose-dependently reduced LDH by (60, 73**%**), CK-MB by (56, 62 %,) and cTnT by (79, 85%) respectively as compared to the DOX group.

Histopathological examination

H&E stained sections of the hearts in the control group (Figure 2A) displayed normal cardiac wall architecture with branching and anastomosing myofibers surrounded by endomysium. The cardiomyocytes with central oval euchromatic nuclei are surrounded with multiple capillaries and fibroblasts. Sections from DOX-treated animals (Fig. 2B) showed focal myocytolysis and vacuolar degeneration of cardiomyocytes with pyknotic (apoptotic) nuclei, congested blood vessels and haemorrhage. On the other hand, HP125 treated group (Fig. 2C) revealed cardiac myofibers reorganization with less myocytolysis, and inflammation, while Hp administration at a dose of 250 mg/kg significantly improved the DOX-induced histological profile (Fig.2D

Effect on oxidant and antioxidants status:

DOX administration produced a significant ($p<0.05$) decrease in GSH, SOD, CAT, GPx by (40, 62, 39, 43%) respectively, and a significant elevation in MDA by (224%) as compared to the control group. Oral administration of HP at 125, 250 mg/kg dose-dependently elevated GSH by (42, 53%), SOD by

(146, 155%), CAT by (41, 59%), GPx by (55, 66%) and reduced MDA by (45, 65%) respectively as compared to DOX group (Figure 3A, B, C, D, E).

Apoptotic markers: Effects on cardiac BCL2, Bax and Caspase 3 gene expression:

Dox treated rats exhibited a significant ($p<0.05$) downregulation in BCL2 by (29%) and a significant upregulation in Bax and Caspase 3 by 66 and 54% respectively, as compared with the control group. Treatment with HP (125, 250 mg/kg) caused a dose-dependent elevation in BCL2 by (58, 89%), and a significant decrease in Bax by (45, 58%), and Caspase 3 by (28, 45%) respectively as compared to DOX group (Figure 4A, B, C).

Effects on cardiac APN, adiponectin receptor1, adiponectin receptor2, AMPKα1, AMPKα2 gene expression:

DOX injection elicited a significant ($p<0.05$) reduction in cardiac gene expression of APN, adipoR1, adipoR2, AMPKα1and AMPKα2 by (19, 24, 29, 44, 36%) respectively, as compared with normal control group. Meanwhile, oral intake of HP (125, 250 mg/kg) caused a dose- dependent elevation in APN by (49 and 87%), adiponectin receptor 1by (104 and 132%), adiponectin receptor 2 by (108, 133%), AMPKα1 by (193, 227%), AMPKα2 by (123, 161%) respectively as compared with DOX group (Figure 5A, B, C, D, E).

Table 1: The primers' sequences of the studied genes

	Forward	Reverse
APN	5′-AATCCTGCCCAGTCATGAAG-3′	5′-TCTCCAGGAGTGCCATCTCT-3'
Adiponectin receptor 1	5′-CGTGGCCTTTATGCTGCTCG-3'	5'-TCTAGGCCGTAACGGAATTC-3'
Adiponectin receptor 2	5'-CCACAACCTTGCTTCATCTA-3'	5'-GATACTGAGGGGTGGCAAAC-3'
AMPKα1	5′-ATCCGCAGAGAGATCCAGAA-3′	5′-CGTCGACTCTCCTTTTCGTC-3′
AMPKα2	5′-GCTGTGGATCGCCAAATTAT-3′	5'-GCATCAGCAGAGTGGCAATA-3'
BCL2	5'-TGTGGATGACTGACTACCTGAACC-3'	5'-CAGCCAGGAGAAATCAAACAGAGG-3'
Bax	5'-CGGCGAATTGGAGATGAACTGG-3'	5'-CTAGCAAAGTAGAAGAGGGCAACC-3'
Caspase 3	5'-GTGGAACTGACGATGATATGGC-3'	5'-CGCAAAGTGACTGGATGAACC-3'
GAPDH	5'-TCAAGAAGGTGGTGAAGCAG-3'	5'-AGGTGGAAGAATGGGAGTTG-3'

Table 2: ECG changes in different groups

group / parameter	Control	Dox	HP 125+DOX	HP 250+DOX
HR (beat/min)	367± 30.7	248.3±28.6*	306.7± 30$^{*\#}$	345± 32$^{\#\$}$
QRS (ms)	35.8±7.1	69.5±10*	56.8±11.2$^{*\#}$	44.8±7.5$^{\#\$}$
PR (ms)	44±6.6	70±6.9*	59.3±6.7$^{*\#}$	50.7±6.5$^{\#\$}$

Results are presented as means± SD (n=6).*P<0.05 compared with control group; #P<0.05 compared with DOX group; $P<0.05 compared with HP125+ DOX group, using one- way ANOVA followed by LSD post hoc test. HP: hypericum perforatum; DOX: doxorubicin**.**

Table 3: Serum levels of LDH, CK-MB and cTnT in different groups:

group / parameter	Control	DOX	HP125+DOX	HP 250+DOX
LDH (U/L)	7.2±1.5	30.4±3.4*	12.3±1.6*#	8.1±1.1#$
CK-MB (U/L)	15.3±2.5	45.6±2.7*	20.4±1.8*#	17.3±2.1#$
cTnT (ng/ml)	1.1±0.5	8.7±0.9*	1.8±0.8#	1.3±0.6#

Results are presented as means± SD (n=6).*P<0.05 compared with the control group; #P<0.05 compared with DOX group; $P<0.05 compared with HP125+ DOX group, using one- way ANOVA followed by LSD post hoc test. HP:hypericum perforatum; DOX:doxorubicin. Lactate dehydrogenase (LDH); Creatine kinase-MB (CK-MB); Cardiac troponin T (cTnT).

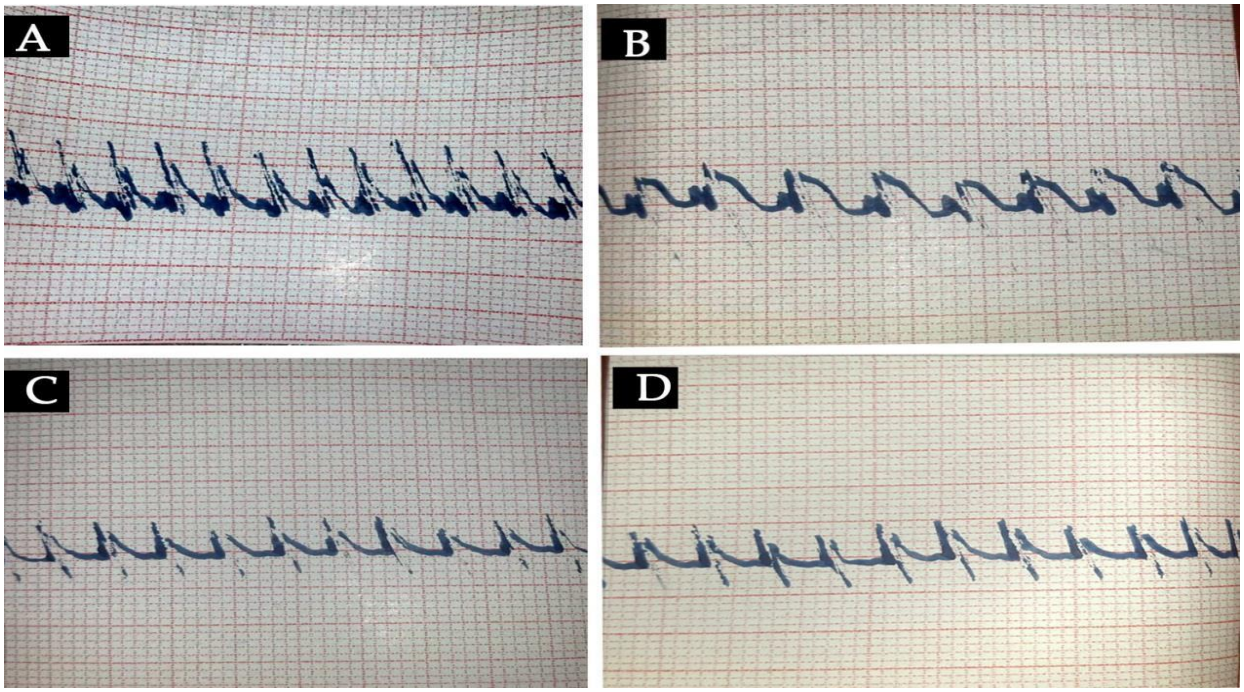

Figure 1: represents E.C.G changes

A: control group reveals normal ECG pattern; B: DOX group reveals bradycardia, wide QRS, prolonged PR interval; C: HP125+ DOX and D: HP250+ DOX groups show increased heart rate, reduction in QRS, PR interval in dose- dependent manner compared to the DOX group.

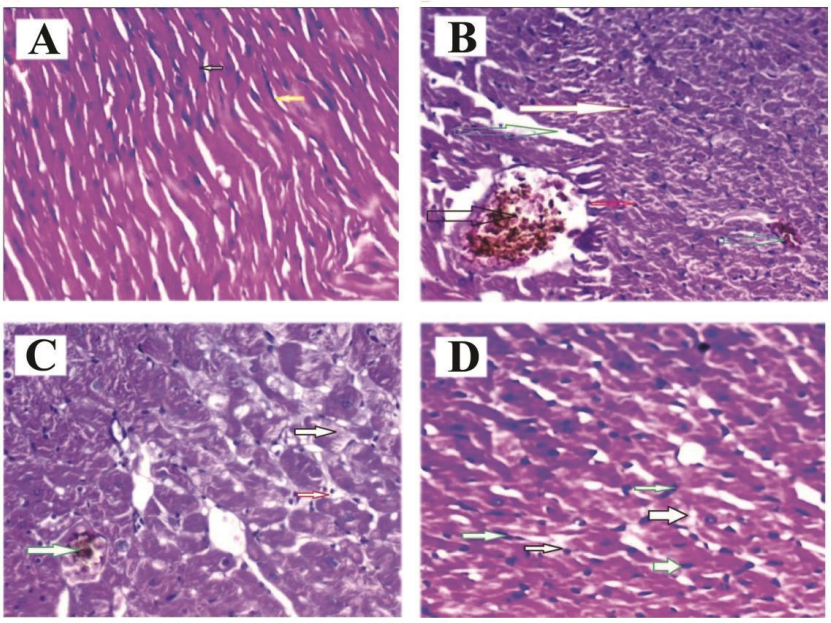

Figure 2: Photomicrographs of the studied groups stained with H&E.

A: Control group: Normal cardiac wall architecture with branching and anastomosing myofibers surrounded by endomysium. The cardiomyocytes have central oval, euchromatic nuclei (black arrow) and surrounded with multiple capillaries and flat nuclei of fibroblasts (yellow arrow). B: DOX-treated group, Focal myocytolysis and vacuolar degeneration of cardiomyocytes (brown arrow) with pyknotic (apoptotic) nuclei (red arrow), congested blood vessels (black arrow), and hemorrhage (green arrow). C: HP 125+ DOX group: Reorganization of cardiac myofibers with less myocytolysis (black arrow), inflammatory cells (red arrow), and congested blood vessels (green arrow). D: HP250+ DOX group: well- organized cardiac myofibers with oval euchromatic nuclei, normal myocardial architecture, marked amelioration of the myocardial nucleic profile. Mild vacuolar degeneration and pyknosis (black arrow) were occasionally detected. HP: Hypericum perforatum; DOX: doxorubicin.

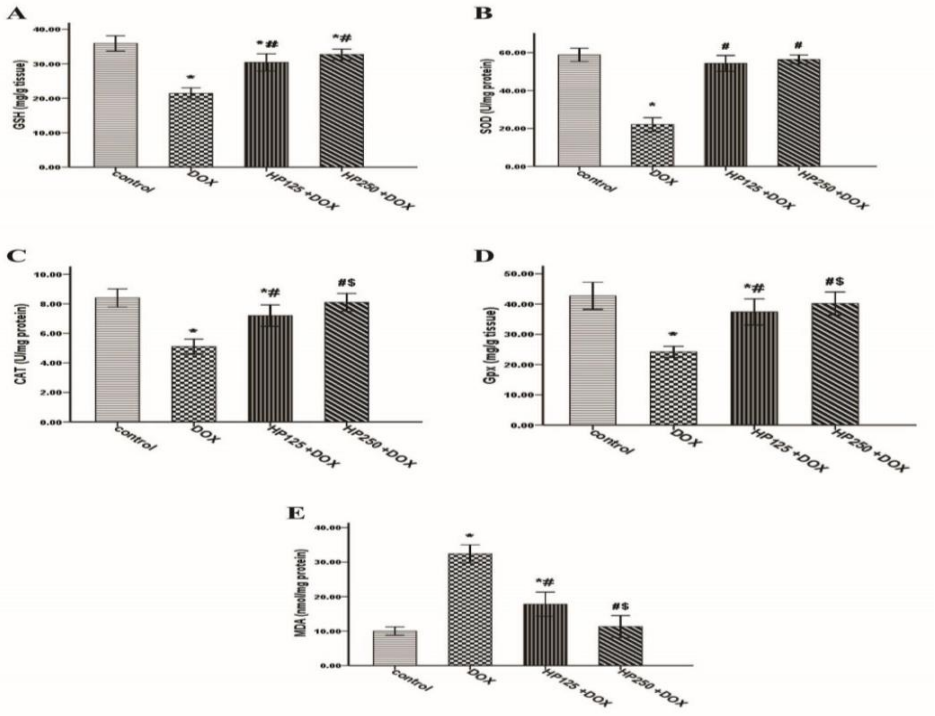

Figure 3: Effect of HP on cardiac GSH, SOD, CAT, GPx, and MDA (3A, B,C, D, and E). Data represent the means of six experiments ± SD; *P<0.05 compared with control; #P<0.05 compared with DOX; $P<0.05 compared with HP125+ DOX, using one-way ANOVA followed by LSD post hoc test. HP: Hypericum perforatum; GSH: reduced glutathione; SOD: superoxide dismutase; CAT: catalase: GPx: glutathione peroxidase; MDA: malondialdhyde; DOX: doxorubicin.

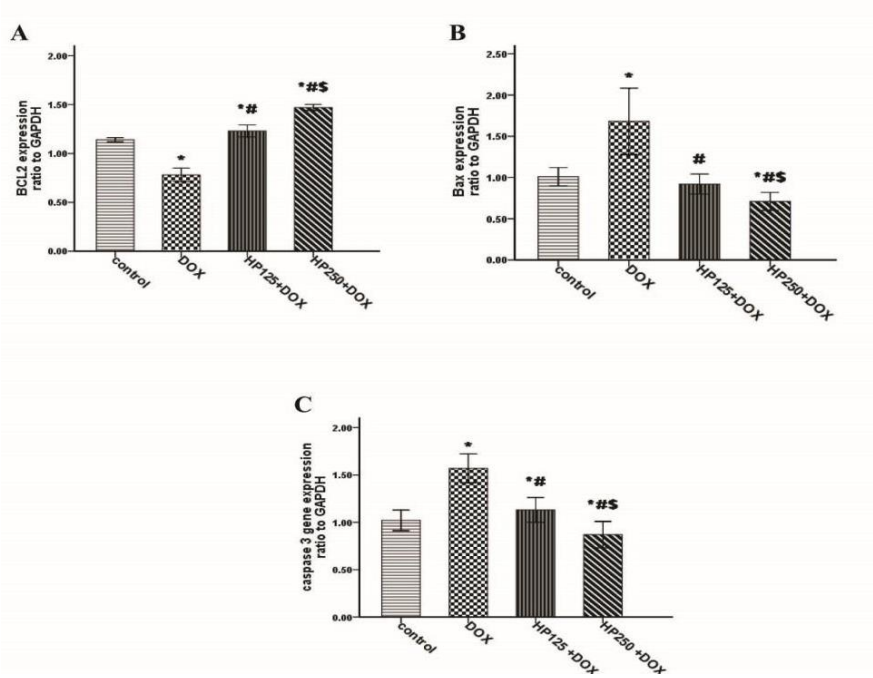

Figure 4: Effect of HP on cardiac BCL2, Bax and Caspase 3 gene expression: 4A, B, and C. Data represent means of six experiments ± SD; *P<0.05 compared with control; #P<0.05 compared with DOX; $P<0.05 compared with HP125+ DOX, using one- way ANOVA followed by LSD post hoc test. HP: hypericum perforatum; DOX: doxorubicin.

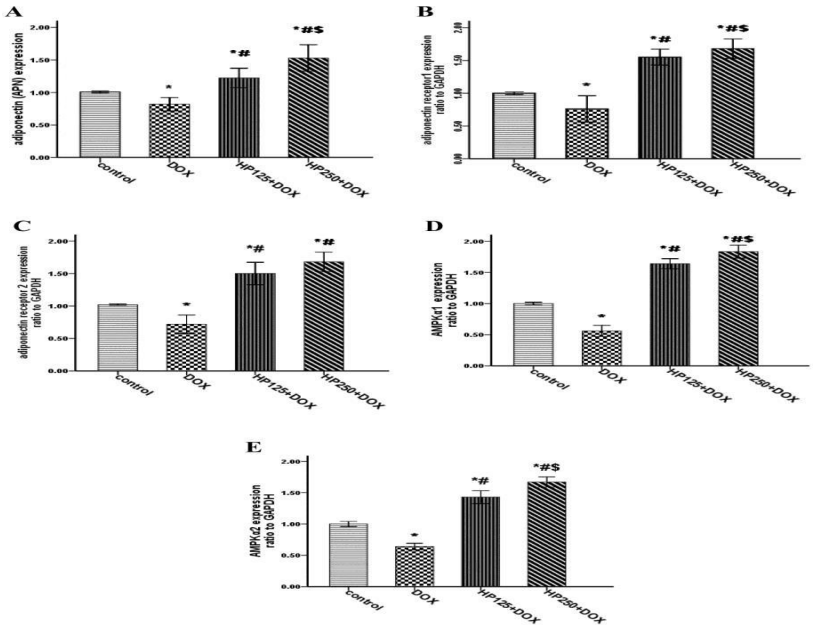

Figure 5: Effect of HP on cardiac APN, adipoR1, adipoR2, AMPKα1, AMPKα2 gene expression: 5A, B, C, D and E). Data represent the means of six experiments ± SD; *P<0.05 compared with control; #P<0.05 compared with DOX;

$P<0.05 compared with HP125+ DOX, using one-way ANOVA followed by LSD post hoc test. HP: hypericum perforatum; APN: adiponectin; adipoR1: adiponectin receptor 1: adipoR2: adiponectin receptor 2; DOX: doxorubicin.

Discussion

Doxorubicin associated cardiotoxicity limits its clinical use **[2]**. Till now, there is no available prophylactic measure to guard against this risk. The current study investigates the protective impact of hypericum perforatum on DOX associated cardiotoxicity via modulation of cardiac adiponectin system. In the current study, DOX altered the ECG pattern, provoked myocytolysis and vacuolar degeneration of cardiomyocytes with pyknotic nuclei which are in accordance with other report **[26**]. Additionally, the serum values of LDH, CK-MB and Cardiac troponin T (cTnT) were elevated in Dox treated rats. LDH and CK-MB are essential enzymes in myocardial metabolism that leaks out with occurrence of cardiac injury. Cardiac troponin is a myocardial regulatory protein, which include 3 types: troponin C, T and I **[27]**. cTnT is expressed only in the myocardium and is present only in the sera of acute myocardial injury patients [**28]**. Therefore, LDH, CK-MB and cTnT serum levels are reliable indicators for myocardial injury. The molecular mechanism of DOX induced cardiotoxicity can be attributed to free radicals overproduction. DOX is reduced by NADPH oxidase with generation of semi-quinone free radicals that interact with oxygen, in the presence of iron, to produce superoxide, hydroxyl, and peroxynitrite free radicals [**29]**. Free radicals in turn cause lipid peroxidation, DNA damage, as well as changes of cellular proteins [**30]**. In this study, the oxidant effect of DOX was demonstrated through the reduced activities of the antioxidant enzymes CAT, GPx, and SOD in addition to diminished GSH levels while there was a robust increase in MDA values. DOX induced oxidative stress provokes the activation of intrinsic apoptotic signaling cascade leading to mitochondrial dysfunction, myofibrillar degeneration, and programmed cell death **[31].** The apoptotic effect of doxorubicin was demonstrated in this study through a decrease in the expression of Bcl2 and increase in the expression of BAX and caspase 3. Similarly, Zhao and Zhang [32], illustrated the apoptotic effect of DOX on cardiomyocytes. On the other hand, the current study showed that HP exerts a protective effect against cardiotoxicity induced by DOX evinced by maintenance of normal cardiac electrophysiology, reduction of cardiac enzymes as well as normal myocardial histoarchitecture. We postulate that the cardioprotective effects of HP can be ascribed to its anti- oxidative and anti-apoptotic characteristics as we found a significant increase in GSH level and CAT, SOD and Gpx activities along with a significant decrease in MDA level in a dose dependent manner in HP treated rats compared to the un-treated group. Our findings are in line with those of Kumar et al. [**33]** who found that HP significantly attenuated the behavioral changes induced in mice by restraint stress via reduction of oxidative stress. Similarly, Zou et al. **[34]** demonstrated that HP administration resulted in a reduction in MDA levels and elevation of SOD and CAT activity in rats fed a high cholesterol diet. DOX-mediated apoptosis is induced by activation of the intrinsic apoptotic signaling pathway. Indeed, the main regulatory elements of apoptosis are BCL-2 family which is divided into anti-apoptotic effectors like BCL-2 and pro-apoptotic effectors like Bax. BCL-2 anti-apoptotic protein, has the ability to prevent the release of cytochrome c and inhibit the apoptotic cell death. On the contrary, apoptotic stimuli increase the expression of Bax and induce conformational changes that inhibit the anti-apoptotic activity of BCL-2, promotes the release of cytochrome c and ultimately initiates apoptosis **[35]**. Mitochondria cytochrome c is released to the cytoplasm and activates caspase 9, which in turn activates the caspase 3 proteins resulting in cell apoptosis **[32]**. The current results revealed that HP administration reduced BAX and caspase 3 gene expression, while enhanced BCL-2 expression when compared to DOX treated animals. We suppose that the antiapoptotic effect of HP may

attribute to the activation of adiponectin signaling pathway. Indeed, adiponectin (APN) belongs to the adipokine family that have an essential role in regulating body mass and metabolism **[36]**. White adipose tissues are primary source of APN. Its serum level is reported to be inversely associated the body mass index [**37]**. APN is demonstrated to be synthesized and produced by murine and human cardiac cells. Wang and Scherer **[38]** reported that APN production by cardiomyocytes is associated with modulation of cardiac function and metabolism. In this study, HP treated groups revealed an up-regulation of cardiac APN and its receptors in a dose dependent fashion. These results are in parallel with Fuller and co-workers **[39**], as they demonestrated that administration of SJW for two-week could boost the expression of APN in epididymal adipose tissue in vivo. In contrast to our results, extracts from SJW's leaves and flowers were shown to suppress the differentiation of adipocytes **[40]**. The reason for this discrepancy is that their study was in vitro, and they used SJW extract with different compositions and concentrations than ours. There was a negative correlation between the expression levels of APN and the cardiovascular, cerebrovascular, and metabolic disorders, suggesting that APN plays pivotal role in the regulation of cardiovascular system **[41]**. High levels of APN decrease the incidence of myocardial infarction (MI) [**42]**, while, a continuous drop in APN level after MI could be an indicator for unfavorable post MI cardiac events **[43]**. Nanayakkara et al. **[44]** stated that APN protected against acute cardiac injury. Additionally, in hypertensive patients, decreased APN values have been linked to progression of left ventricular hypertrophy **[45]**. Indeed, adiponectin, through activating the adiponectin receptors (adipoR1 and adipoR2), can trigger a number of intracellular signaling cascades including AMPK [**46]**. Zhao et al. **[47]** suggested that APN protects mesenchymal stem cells of bone marrow against flow shear stress by activating AMPK, which stimulates phosphorylation of acetyl CoA carboxylase (ACC) and enhances Bax down-regulation and BCL2 upregulation. This was confirmed by our study as there was an up-regulation of APN receptors and increase in APN levels, upregulation of AMPK α1 and α2, up- regulation of BCL-2 and down regulation of BAX and caspase 3 gene expressions in HP treated groups when compared to DOX-treated group. In the present study, there was up-regulation of AMPK gene expression in HP treated groups. Indeed, AMPK is the main coordinator of cardiac metabolism. AMPK activation regulates glucose, fatty acids, and cholesterol metabolism, in addition to cell growth and apoptosis **[48].** AMPK activation is also beneficial for preventing myocardial injury in many conditions such as heart failure, cardiac fibrosis and myocardial ischemia **[49]**.

In conclusion, the present study supposes HP as a potential promising agent that can ameliorate the cardiotoxicity of DOX through preserving the cardiac adiponectin-AMPK signaling. Future studies on HP are recommended to support its potential use in preventing myocardial damage in various cardiovascular disorders.

Conflicts of interests

There are no disclosed conflicts of interest

Acknowledgement

The authors acknowledge Dr. Hayam E. Rashed, pathology department, faculty of medicine of Zagazig university for performing the pathological examination.

Authors' contribution statement: Nisreen E. Elwany conceived and designed research. Nisreen E. Elwany, Nevertyty Mohamed Mahmoud, Amira Mohamed Abdelhamidconducted experiments. Nisreen E. Elwany, Amira Mohamed Abdelhamid analyzed data. Nisreen E. Elwany, Nevertyty Mohamed Mahmoud, Amira Mohamed Abdelhamidwrote the manuscript. Nisreen E. Elwany, Nevertyty Mohamed Mahmoud read and approved the manuscript.

References

1. Pugazhendhi A, Edison TNJI, Velmurugan BK, et al., Toxicity of Doxorubicin (Dox) to

different experimental organ systems. Life Sci.2018; 1(200): 26-30.

2. Zhang D, Xu Q, Wang N, et al., A complex micellar system co- delivering curcumin with doxorubicin against cardiotoxicity and tumor growth. Int J Nanomedicine. 2018; 10(13):4549-4561.

3. Danz E D, Skramsted J, Henry N,et al., Resveratrol prevents doxorubicin cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway. Free Radic. Biol. Med.2009; 46:1589–1597.

4. Xiong C, Wu YZ, Zhang Y, et al., Protective effect of berberine on acute cardiomyopathy associated with doxorubicin treatment. Oncol Lett. 2018; 15(4): 5721-5729.

5. Hui X, Lam KS, Vanhoutte PM, Xu A. Adiponectin and cardiovascular health: an update. BrJPharmacol. 2012 Feb;165(3):574-90. doi:10.1111/j.1476-5381.2011.01395.x. PMID: 21457225; PMCID: PMC3315032.

6. Yamaguchi T, Kitamori K, Ichihara G, et al., Serial changes in adipocytokines and cardiac function in a rat model of the metabolic syndrome. Clin Exp Pharmacol Physiol. 2013; 40(7):443-8.

7. Paterniti I, Briguglio E, Mazzon E, et al., Effects of Hypericum Perforatum, in a rodent model of periodontitis.BMC Complement Altern Med.2010; 23: 10-73.

8. Jenke A, Schur R, Röger C, et al., Adiponectin attenuates profibrotic extracellular matrix remodeling following cardiac injury by up-regulating matrix metalloproteinase 9 expression in mice. Physiol Rep. 2017; 5(24): e13523.

9. Lu YH, Du CB, Liu JW, et al., Neuroprotective effects of Hypericum perforatumon trauma induced by hydrogen peroxide in PC12 cells. Am. J.Chin.Med.2004; 32: 397–405.

10. Zou YP, Lu YH and Wei DZ. Protective effects of a flavonoid- rich extract of Hypericum perforatum L. against hydrogen peroxide-induced apoptosis in PC12cells. Phytother. Res 2010; 24 (1):S6–S10.

11. Maruyama S, Shibata R, Ohashi K, et al., Adiponectin ameliorates doxorubicin induced cardiotoxicity through Akt protein-dependent mechanism. J Biol Chem. 2011; 286(37):32790-800.

12. Konishi M, Haraguchi G, Ohigashi H, et al., Adiponectin protects against doxorubicin-induced cardiomyopathy by anti- apoptotic effects through AMPK up-regulation. Cardiovasc Res. 2011;89 (2):309-19 .

13. Ng QX, Venkatanarayanan N and Ho CY. Clinical use of Hypericum perforatum (St John's wort) in depression: A meta- analysis.J Affect Disord. 2017; 1(210):211-221.

14. Cao Z, Wang F, Xiu C, et al., Hypericum perforatum extract attenuates behavioral, biochemical, and neurochemical abnormalities in Aluminum chloride-induced Alzheimer's disease rats. Biomed Pharmacother. 2017; 91:931-937.

15. Cakir M, Duzova H, Baysal I, et al., The effect of hypericum perforatum on kidney ischemia/reperfusion damage. Ren Fail. 2017; 39(1): 385-391.

16. You MK, Rhuy J, Jeong KS, et al., Effect of St. John's Wort (Hypericum perforatum) on obesity, lipid metabolism and uterine epithelial proliferation in ovariectomized rats. Nutr Res Pract.2014;8(3):292-6.

17. Tian JY, Tao RY, Zhang XL, et al., Effect of Hypericum perforatum L. extract on insulin resistance and lipid metabolic disorder in high-fat-diet induced obese mice. Phytother Res. 2015; 29(1):86-92 .

18. de Oliveira JS, Pinto ME, Santana LA, et al., Biological Effects of Medicinal Plants on Induced Periodontitis: A Systematic Review.

International Journal of Dentistry. 2016; 3719879 .

19. Kaplan HM , Izol V, AridoganIA, et al., Protective Effect of Hypericum perforatum Extract on Gentamicin Induced Nephrotoxicity in Mice. International Journal of Pharmacology. 2016. 12(6):663-668.

20. Abd El Motteleb DM and Abd El Aleem DI. Renoprotective effect of Hypericum perforatum against diabetic nephropathy in rats: Insights in the underlying mechanisms. Clin Exp Pharmacol Physiol., 2017; 44(4):509-521 .

21. Hatano T, Sameshima Y, Kawabata M, et al., St. John's wort promotes adipocyte differentiation and modulates NF-κB activation in 3T3-L1 cells. Biol Pharm Bull.2014; 37(7): 1132-8.

22. Mantawy EM, El-Bakly WM, Esmat A, et al., Chrysin alleviates acute doxorubicin cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Eur J Pharmacol. 2014; 5(728): 107-18.

23. El-Bakly WM and Hasanin AH. Hypericum Perforatum Decreased Hippocampus TNF-α and Corticosterone Levels with No Effect on Kynurenine / Tryptophan Ratio in Bilateral Ovariectomized Rats. Korean J Physiol Pharmacol.2014; 18(3): 233-9 .

24. Van Acker SA, Kramer K, Voest EE, et al., Doxorubicin-induced cardiotoxicity monitored by ECG in freely moving mice. A new model to test potential protectors. Cancer Chemother Pharmacol.1996; 38(1):95-101.

25. Bancroft J and Stevens A.Theory and Practice of Histological Techniques. In: Stevens A (editor). New York: Churchill Livingstone, 1982 , 188-90.

26. Abdel-Daim MM, Kilany OE, Khalifa HA et al., Allicin ameliorates doxorubicin- induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Cancer Chemother Pharmacol. 2017; 80(4):745-753 .

27. Sze J, Mooney J, Barzi F, et al., Cardiac troponin and its relationship to cardiovascular outcomes in community populations – a systematic review and meta-analysis. Heart Lung Circ. 2016; 25: 217–228.

28. Sun Z, Yan B, WY Y, et al., Vitexin attenuates acute doxorubicin cardiotoxicity in rats via the suppression of oxidative stress, inflammation and apoptosis and the activation of FOXO3a. Exp Ther Med.2016; 12:1879–1884.

29. Cappetta D, De Angelis A, Sapio L, et al., Oxidative Stress and Cellular Response to Doxorubicin: A Common Factor in the Complex Milieu of Anthracycline Cardiotoxicity. Oxid Med Cell Longev. 2017; 1521020 .

30. Gaschler MM and Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017; 482(3):419-425 .

31. Shaker RA, Abboud SH, Assad HC, et al., Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis. BMC Pharmacol Toxicol. 2018; 10(19):1-3.

32. Zhao L and Zhang B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. Sci Rep.2017; 16(7):44735.

33. Kumar A, Garg R and Prakash AK. Effect of St. John's Wort (Hypericum perforatum) treatment on restraint stress- induced behavioral and biochemical alteration in mice. BMC Complement Altern Med. 2010; 7, 10, 18. doi:

10.1186/1472-6882-10-18.

34. Zou Y, Lu Y and Wei D. Hypocholesterolemic effects of a flavonoid-rich extract of Hypericum perforatum L. in ratsfed a cholesterol-rich diet. J Agric Food Chem. 2005; 53(7):2462-6.

35. Renu K, VG A, PB TP et al., Molecular mechanism of doxorubicin-

induced cardiomyopathy - An update.Eur J Pharmacol. 2018; 5(818):241-253.

36. Esfahani M, Movahedian A, Baranchi M et al., Adiponectin: anadipokine with protective features against metabolic syndrome. Iran J Basic Med Sci. 2015; 18(5): 430-42.

37. Cohen SS, Gammon MD, Signorello LB, et al., Serum adiponectin in relation to body mass index and other correlates in black and white women. Ann Epidemiol.2011; 21(2): 86-94 .

38. Wang ZV and Scherer PE. Adiponectin, the past two decades. J Mol Cell Biol.2016; 8(2):93-100.

39. Fuller S, Richard AJ, Ribnicky DM, et al., St. John's Wort Has Metabolically Favorable Effects on Adipocytes in Vivo, Evid Based Complement Alternat Med. 2014; 862575

40. Amini Z, Boyd B, Doucet J, et al., St. John's Wort inhibits adipocyte differentiation and induces insulin resistance in adipocytes. Biochem Biophys Res Commun. 2009; 388(1):146-9 .

41. Ghantous, C. M., Azrak, Z., Hanache, S., et al. Differential Role of Leptin and Adiponectin in Cardiovascular System. Int J Endocrinol. 2015; 534320 .

42. Souza RA, Alves CMR, de Oliveira CSV, et al., Circulating levels of adiponectin and extent of coronary artery disease in patients undergoing elective coronary angiography. Braz J Med Biol Res. 2017; 51(2):6738.

43. Sharma S, Colangelo LA, Lloyd- Jones D, et al., Longitudinal associations between adiponectin and cardiac structure differ by hypertensive status: coronary artery risk development in young adults (CARDIA). Cardiovasc Endocrinol. 2016; 5:57–63.

44. Nanayakkara G, Kariharan T, Wang L, et al., The cardio- protective signaling and mechanisms of adiponectin. Am J Cardiovasc Dis. 2012; 2(4):253-66.

45. Caselli C, D'Amico A, Cabiati M, et al., Back to the heart: the protective role of adiponectin. Pharmacol Res.2014; 82: 9-20 .

46. Wang Y, Ma XL and Lau WB. Cardiovascular Adiponectin Resistance: The Critical Role of Adiponectin Receptor Modification. Trends Endocrinol Metab. 2017; 28(7):519-530 .

47. Zhao L, Fan C, Zhang Y, et al., Adiponectin enhances bone marrow mesenchymal stem cell resistance to flow shear stress through AMP-activated protein kinase signaling. Sci Rep., 2016; 15(6):28752.

48. Jeon SM. Regulation and function of AMPK in physiology and diseases. Exp Mol Med. 2016; 48(7): 245.

49. Daskalopoulos EP, Dufeys C, Beauloye C, et al., AMPK in Cardiovascular Diseases. EXS. 2016; 107:179-201.