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The Impact of NF-κB and Keap1/Caspase-3 Signaling Pathway on protective Effects of sitagliptin in a Rat Model of Doxorubicin-Induced Cardiac Dysfunction

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- cardiac toxicity,
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- caspase-3

Abstract

Background: Doxorubicin (DOX) treatment is a primary cause of chemotherapy-induced cardiotoxicity. Antidiabetic sitagliptin (STG) has been shown to improve cardiovascular functions through anti-inflammatory, antioxidant, and anti-apoptotic effects**.** Oxidative stress plays a crucial role in the development of various cardiovascular diseases, especially those related to endothelial dysfunction. The transcription factor nuclear factor erythroid-2-like 2 (Nrf2) is the main regulator of antioxidant defense mechanisms. Nrf2, along with its suppressor protein Kelch-like ECH-associated protein 1 (Keap1), controls the levels of reactive oxygen species (ROS). **Objective:** This study aimed to evaluate the role of the Nrf2/caspase-3 signaling pathway in the cardiotoxic effect of DOX and the potential role of sitagliptin (STG). **Materials and Methods:**Rats were divided into four groups, each comprising eight animals (n=8): a control group, an STG group (receiving 10 mg/kg/day for 21 days), a DOX group (receiving 1mg/kg i.p per day for five injections \ week for 3 weeks) and STG+DOX group (treated by STG and DOX as previously mentioned). **Results:** The effect of DOX on cardiac tissue detected a significant reductionin ABP and deviated ST segment, while STG improved DOX-induced cardiotoxicity markers, oxidative stress, and inflammatory markers. Also, STG regulates cardiovascular hemodynamics and reduces both cardiac caspase-3 and Keap-1 and upregulates NrF2 gene expression levels. **Conclusion:** STG administration can exert protective effects against doxorubicin-induced cardiac toxicity through anti-inflammatory, antioxidant, and anti-apoptotic properties, in addition to promoting the Nrf2 signaling pathway.

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Introduction

DOX is a chemotherapy drug that is commonly used to treat various types of cancers, including ovarian, thyroid, gastric, leukemia, and breast cancers **[1].** Although Dox has been proven to increase the survival rate of cancer patients, Dox-induced cardiac toxicity (DIC) has been recognized as a complication of chemotherapy. Patients with childhood cancer and those treated with DOX have a high risk of developing symptomatic cardiac dysfunction at an early stage, and this risk remains high for years after treatment **[2].**

The antitumor activity of doxorubicin, through direct DNA damage, interferes with the function of many enzymes necessary for DNA replication **[2].** The mechanisms to explain doxorubicin-induced cardiotoxicity are not completely clear. It seems to be a multi-step process, with different potential pathways involved that lead to cardiomyocyte death **[3].** The main mechanisms that have been proposed by various research groups include oxidative stress, lipid peroxidation, Ca2+ homeostasis dysregulation, iron metabolism defect, gene expression modulation, and activation of several programmed cell death pathways leading to cardiomyocyte loss**.** Therefore, doxorubicin causes severe cardiotoxicity that is dose-dependent and frequently leads to dilated cardiomyopathy, systolic cardiac dysfunction, and, in severe cases, heart failure, all of which have a major detrimental effect on the patient's health and present a major obstacle to the clinical use of doxorubicin. Various strategies have been tested in both the prevention and treatment of doxorubicin-induced cardiotoxicity to improve the quality of life of cancer survivors **[4].**

STG is a highly selective DPP-4 inhibitor. Its major application is in the treatment of type 2 diabetes **[5].** It has an anti-diabetic effect on the blood by preventing the DPP4 enzyme from degrading glucagon-like peptide 1 (GLP-1), GLP-1 analogs have recently been demonstrated to be helpful during ischemia-reperfusion (I/R) injury in both clinical and animal models by improving cardiac function, enhancing myocardial glucose uptake, and limiting infarct size. increase of myocardial glucose uptake and improvement in cardiac function.

STG exerts a protective effect on the heart function. It has been found to protect the heart from ROS. It has an important role in the regulation of defensive genes activation and induction of antioxidant enzymes such as SOD and glutathione peroxidase leading to suppression of injury evoked by ROS and protection of cells against oxidative stress injurious effects **[6].**

The role of caspases in programmed cell death has been reviewed many times. It is widely accepted that, in mammals, two main pathways have evolved to activate the caspase cascade. The mitochondrial pathway (intrinsic pathway) and the death receptor pathway (extrinsic pathway). The release of pro-apoptotic proteins, including cytochrome c, from the inter-membrane space into the cytoplasm **[7].** Cytochrome c can then bind Apaf-1, forming the apoptosome and activating caspase-9. Once active, caspase-9 can directly cleave and activate caspase-3 and caspase-7. **[8]**. This study was designed to demonstrate the effect of STG on DOX-induced cardiotoxicity in adult male rats.

2. MATERIAL AND METHODS:

2.1. Chemical used:

DOX, provided as ampoules (D-RUBILEE-50 ampoules) (by Magicine Pharma CO., New Delhi, India. Lot No: DXLL18B24-B),

STG, provided as tablets (JANUVIA 100mg tablets) (by Merck Sharp and Dohme Company, Northumberland, UK. Lot No: S008904.

Urethane: was purchased from Urethane and sourced from Sigma-Aldrich (St. Louis, MO, USA). It was dissolved in saline before use.

2.2. Animals:

This experimental study was achieved using 24 adult male albino rats aged above 2 months and withweights between 180 and 220 g. They were obtained from the animal research center, Faculty of Veterinary Medicine, Benha University. Each of the 4 rats was placed in a separate cage and was fed a standard diet, with free access to food and water. They were placed at room temperature (25 ° C) with a 12:12-h light/dark cycle. These conditions were continued for 10 days before the experiment for acclimatization. The study period lasted for 3 weeks. Experimental rats should be under completely healthy conditions all through the experiment and under the care of a professional technician and a qualified researcher. All procedures were approved by the ethical committee of the Benha Faculty of Medicine **(RC 20-12-2024**). No rats died throughout the experiment. At the end of the study, the rats were incinerated at Benha University Hospital incinerator.

2.3. Experimental design:

After one week of the acclimatization period, the rats (n=6) were split into four equal groups. Before beginning treatments, there was no discernible variation in the body weight of the animals in the various groups; the animals were distributed at random. The rats were classified into 4 groups as follows:

Group (I) (Control group), rats were injected with saline intra peritoneal (i.p) (2.5 ml/kg/ day) for 3 weeks **[9].**

Group (II) STG group was given (10 mg/kg/day) orally by esophageal tube for 3 weeks**[10].**

Group (III) (DOX group): rats were injected by DOX in a dose of 1mg/kg i.p per day for five injections \ weeks, this is for 3 weeks to produce a cumulative dose of 15mg/kg **[11].**

Group (IV) (STG +DOX group): Thesame dose of STG and DOX were given as mentioned before.

2.4. Procedure of the experiments:

After a 12-hour post-treatment period and an overnight fast, rats were given 1.5 g/kg of urethane to induce anesthesia so that their cardiac condition could be monitored by electrocardiogram. The supplier of urethane was Sigma-Aldrich, located in St. Louis, Missouri, USA. The SBP, DBP, Mean BP, and ECG of each rat were recorded at the end of the experiment, then the animals were fixed on an operating table. A craniocaudal incision of about 2 cm was made, parallel with and slightly to the left of the sternum. The blood samples were taken from the aorta, then put into non-heparinized tubes and allowed to clot. The resulting sera were separated using automated pipettes and stored in Eppendorf tubes at -20°C for subsequent biochemical analysis. Then the hearts were exercised, washed, and stored. After collecting blood samples, light pressure on the thorax helps to bring the heart outside without damage. The heart was divided longitudinally into two halves. Half was immediately washed with normal saline, frozen in liquid nitrogen, and stored

at -80°C. The other half was kept in formaldehyde to be stained with Hematoxylin and Eosin (H&E) and examined for histopathological lesions in the heartsby using a light microscope (Olympus BX-50 Olympus Corporation, Tokyo, Japan).

2.5 ECG and ABP recording:

Typically, for Lead II, the negative electrode should be placed on the right forelimb, the positive electrode on the left hind limb, and the reference (ground) electrode on the right hind limb or another neutral site.

The program Power Lab 4/20 (data collecting system, AD Instruments Pty Ltd, Australia) was connected to the electrodes. The results were computed automatically based on the millivolts of voltage calibration. Continuous electrocardiogram (ECG) monitoring was done by using standard artifact-free lead II. The ECG was used to record the heart rate (beat/min), T waves (mv), and ST depression (12). ABP was measured by carotid artery cannulation using Software Lab Chart 8 power lab recorder and analyzer (AD Instruments, Mountain View, CA, USA) (13).

2.6. Biochemical assessment:

2.6.1 Assessment of cardiotoxicity markers.

Sera were separated from the clotted blood by centrifugation at 5000 rpm for 10 min and stored at -20°C till the assay ofCK-MB (a highly reliable biomarker of cardiac injury). It was detected based on a kinetic photometric method using an available commercial kit (Elitech, France). LDH activity was estimated according to standard methods using an available commercial kit (Human Gesllschaft fur Biochemica und Diagnostica, Germany).

2.6.2 Assessment of oxidative stress and inflammatory mediators

Serum SOD activity was determined using a commercial kit Provided by (Kamiya Biomedical Company, California, U.S.A. Lot No: KT-745). Serum malondialdehyde (MDA) was assessed using commercial kits provided by (Abcam Biochemicals, Cambridge Science Park, Cambridge, UK, Lot No: ab118970).

Heart tissue was homogenized in a pH 6–7 phosphate buffer. For fifteen minutes, the homogenized tissue was centrifuged at 10,000 g and 4 °C. The supernatant was utilized to assess the level of TNF-α. TNF-α was measured using a Rat TNF-α ELISA kit (CODE: ELR-TNFα-1; Ray Biotech. Whereas, inflammatory mediators Nuclear Factor Kappa B (NF-kB), was measured using ELISA Kit (Catalog # E-EL-R0674; Elabscience, Texas, USA),

2.6.3 Determination of relative values of cardiac gene expression for caspase-3, NrF2 mRNA, and Keap-1

Total RNA for caspase-3, Keap -1, and NrF2 was extracted from cardiac tissues using a GF-l isolation kit (vivantis, Malasyia). Following the manufacturer's instructions, RNA concentration was measured at 260 nm, and purity was assessed using the absorbance ratio at 260/280 nm with a NanoPhotometer. The QuantiTect reverse transcription kit (Qiagen Valencia, USA) was used to convert 1 μg of total RNA into single-stranded complementary DNA (cDNA)(14). The mRNA levels of NrF2, Keap-1, and caspase-3 in rat cardiac tissues were quantified using the SensiFAST SYBR® No-ROX Kit (Bioline, USA). Rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a housekeeping gene and internal control. Gene-specific PCR primers (Table 3) were designed using Primer Express 3.0 (Applied Biosystems, USA) based on nucleotide sequences from the Gene Bank. Expression levels were calculated using the standard 11Ct method.

Table 1: Primer sequences and amplicon lengths for Nrf2, Caspas-3, and Keap1in the target-site PCR for high-throughput sequencing.

Gene	primer sequence	
GAPDH	Forward	5'-TGC CAC TCA GAA GAC TGT GG-3'
	Reverse	5'-GGA TGC AGG GAT GAT GTT CT-3'
Caspase-3	Forward	5'-GGC CGA CTT CCT GTA TGC TT-3'
	Reverse	5'-CGT ACA GTT TCA GCA TGG CG-3'
Keap	Forward	5′-CAGCCCTGTACTCTTCCCTCCTT-3′
	Reverse	5′-TGCATACATCACCGCGTCCC-3′
NrF2	Forward	5'-CAG TCT TCA CCA CCC CTG AT-3'
	Reverse	5'-TTG CTC CAT GTC CTG CTG TA-3'

Nrf2, nuclear factor erythroid-2-like factor -2, GAODH: glyceraldehyde 3-phosphate dehydrogenase, Keap1, Kelch-related

2.6.4 Histopathological examination:

Cardiac tissue samples were immersed in 10% formalin for 48 hours to fixate. Thereafter, 4 μm thick sections were meticulously prepared, processed, and stained using hematoxylin and eosin to examine the histological architecture of the myocardial fibers in detail. The sections were stained with hematoxylin and eosin (H&E) for standard histological analysis and Masson trichrome (MT) to assess collagen fibers, following the procedure outlined by Bancroft and Layton (2013). Slide examination and histomorphometric measurements were conducted using a light microscope (Olympus-Bx; 4500) fitted with a digital camera (Nikon-Coolpix; 4500), with objective lenses magnified at ×10 and ×40.

3. Statistical analysis:

The data were analyzed using the program: Statistical Package for Social Science (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA, 2000). In the statistical comparison between the different groups, the significance of difference was tested using a one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test for comparison between every two groups. The p-values < 0.05 were considered statistically significant.

4. RESULTS:

4.1. STG improved DOX-induced cardiotoxicity markers in rats:

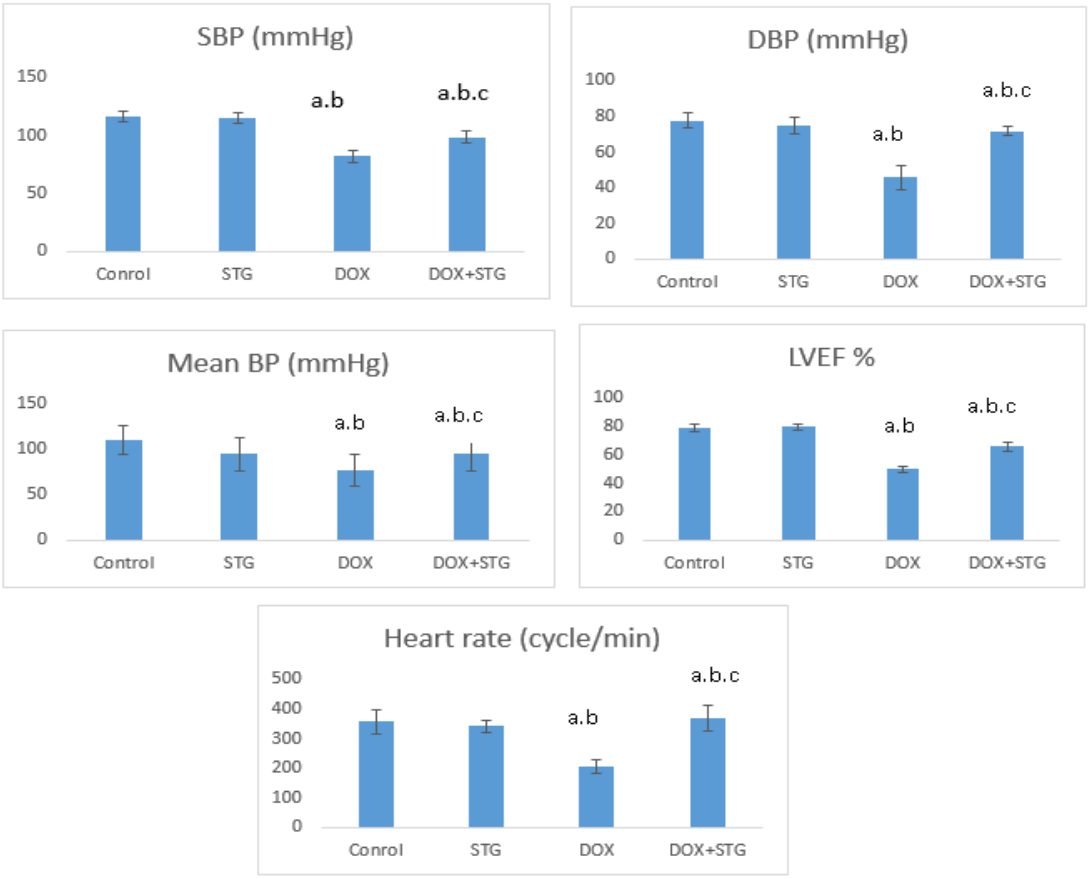
According to our findings, Rats injected with DOX (Dox group) showed cardiac tissue injury with elevated serum CK-MB and LDH ($P<0.05$) in the DOX group compared with the STG group and control group. The rise in these parameters was dramatically inhibited by STG pretreatment in the STG+DOX group (table 2). In contrast, STG ameliorated the DOX-induced myocardial injury, as indicated by the remarkable reversal of the ST segment (Figure1).

Table (2): Evaluation of cardiac enzymes

Groups	Control group(I)	STG group (II)	DOX group (III)	DOX + STG group (IV)
CK-MB(U/L)	314.67 ± 35.16	311.567 ± 34.15	602.83 ± 37.15[a, b]	317.54 ± 31.16 [c]
LDH (μ/U)	197 ± 15.28	199 ± 14.18	318 ± 26.75[a,b]	201.17 ± 18.50 [c]

DOX: Doxorubicin; STG: sitagliptin; CK-MB: Creatine phosphokinase; LDH: lactate dehydrogenase, Data are represented as Mean ± SD., n=8. $P < 0.05$ is significantly tested by ANOVA and post hoc multiple comparison LSD method. a: $P < 0.05$ vs. Control group; b: $P < 0.05$vs STG group, c: $P < 0.05$vs. DOX group

Figure (1): Evaluation of SBP, DBP, Mean ABP, Heart rate and LVEF%



DOX: Doxorubicin; STG: sitagliptin; SBP; systolic blood pressure, DBP; diastolic blood pressure, LVEF %; left ventricular ejection fraction percent. Data are represented as Mean ± SD., n=8. P < 0.05 is significantly tested by ANOVA and post hoc multiple comparison LSD method. a: P < 0.05 vs. Control group; b: P < 0.05vs STG group, c: P < 0.05vs. DOX group

4.2. STG improved the DOX-induced cardiac oxidative stress and inflammation in rats.

Compared to the control group, DOX injection resulted in oxidative stress, which was detected by a substantial rise in cardiac MDA in DOX (P<0.05). Notably, rats that were administrated with STG before DOX injection in group STG+DOX showed a reduction in the MDA level. In contrast, rats in group III that were given DOX exhibited a substantial decrease in SOD levels when compared to the control and STG groups (P<0.05). Serum SOD was significantly improved by the combination of DOX and STG compared to the DOX group (P<0.05) (table 3).

Table (3): Assessment of oxidative stress and inflammatory markers

Groups	Control group (I)	STG group (II)	DOX group (III)	DOX + STG group (IV)
SOD (U/mL)	14.13 ± 2.97	15.22± 2.97	6.32 ± 1.47 [a,b]	19.70 ± 1.84
MDA (nmoL/mL)	1.90 ± 0.40	1.95± 0.30	3.21 ± 0.60 [a,b]	1.26 ± 0.24 [a,b,]
TNF-α (pg/ mg protein)	1.95± 0.58	2.234 ± 0.61	7.33 ± 2.07[a,c]	2.3 ± 0.19[a,b,c]
NF-KB (pg/mg protein)	58.52± 4.52	58.54 ± 5.70	168.37 ± 4.87 [a,b]	85.67 ± 16.56 [a, b, c]

DOX: Doxorubicin; STG: sitagliptin; SOD: superoxide dismutase enzyme. MDA; Malondialdehyde, TNF-α: tumor necrotic factor -α, NF-KB: nuclear factor-kappa B, Data are represented as Mean ± SD., n=8. P < 0.05 is significantly tested by ANOVA and post hoc multiple comparison LSD method. a: P < 0.05 vs. Control group; b: P < 0.05vs STG group, c: P < 0.05vs. DOX group

4.3. STG ameliorates DOX-induced cardiac inflammatory markers

One of the main causes of the death of cardiomyocytes and the ensuing heart failure is inflammation. The levels of the proinflammatory cytokines TNF-α in cardiac tissue and the expression of the NF-KB gene were examined for this in (table 3). When DOX was injected into rats, the inflammatory mediators TNF-α significantly increased compared to the control and STG-receiving rats. The combination of DOX and STG significantly reduced TNF-α (P<0.05), suggesting that STG has anti-inflammatory properties. NF-KB was dramatically elevated in the DOX-treated group as compared to the control and STG groups (P<0.05). NF-KB was significantly reduced when STG and DOX were combined, suggesting that STG has a beneficial anti-inflammatory effect.

4.4 STG alleviates cardiovascular hemodynamics associated with DOX-induced myocardial injury.

When comparing the DOX group to the control and STG groups, our study found a significant increase in ST-segment amplitude (P<0.05). The ST amplitude significantly decreased after STG therapy with DOX, with a noteworthy drop-in heart rate in the Dox group compared to the control and STG groups (P < 0.05). SBP, DBP, and Mean BP all significantly decreased (P<0.05), which was used to measure the cardiac dysfunction of DOX. The DOX+STG group showed a significant improvement in blood pressure measures (P<0.05) (figure 2).

DOX-induced bradycardia in rats with a marked decrease in heart rate compared to control rats (P<0.05) while STG administration in combination with DOX improves the heart rate. Regarding LVEF% %, which represents the volume of blood pumped by the cardiac chambers and the ventricular systolic function, it was notably diminished in the DOX group compared with the control group (P<0.05). STG improved this deterioration in LVEF in rats who received STG with DOX (figure 3).

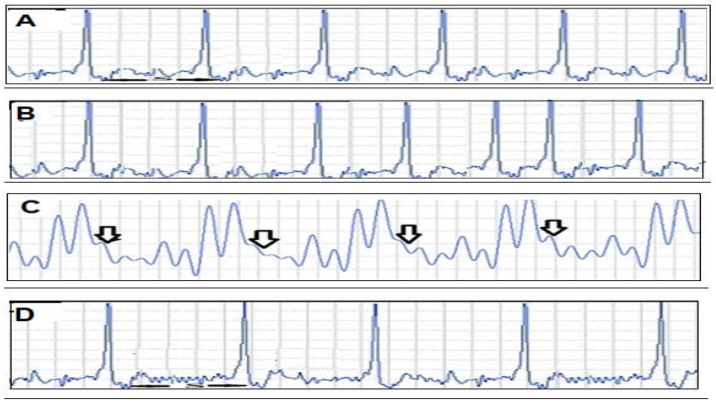
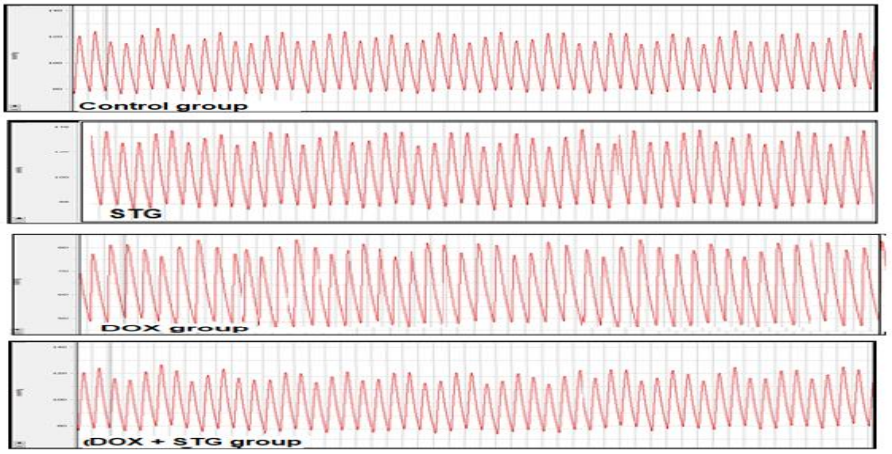


Figure 2: Lead II ECG in the studied groups

A. Control group normal ST segment height
B. STG group with normal ST segment
C. DOX group showing notched R wave and elevated ST segment (black arrow).
D. DOX + STG group showing normal waves and normal ST segment height.

Figure (3): ABP in different studied groups: Control and STG groups showed normal SBP and DBP while DOX group showed hypotension



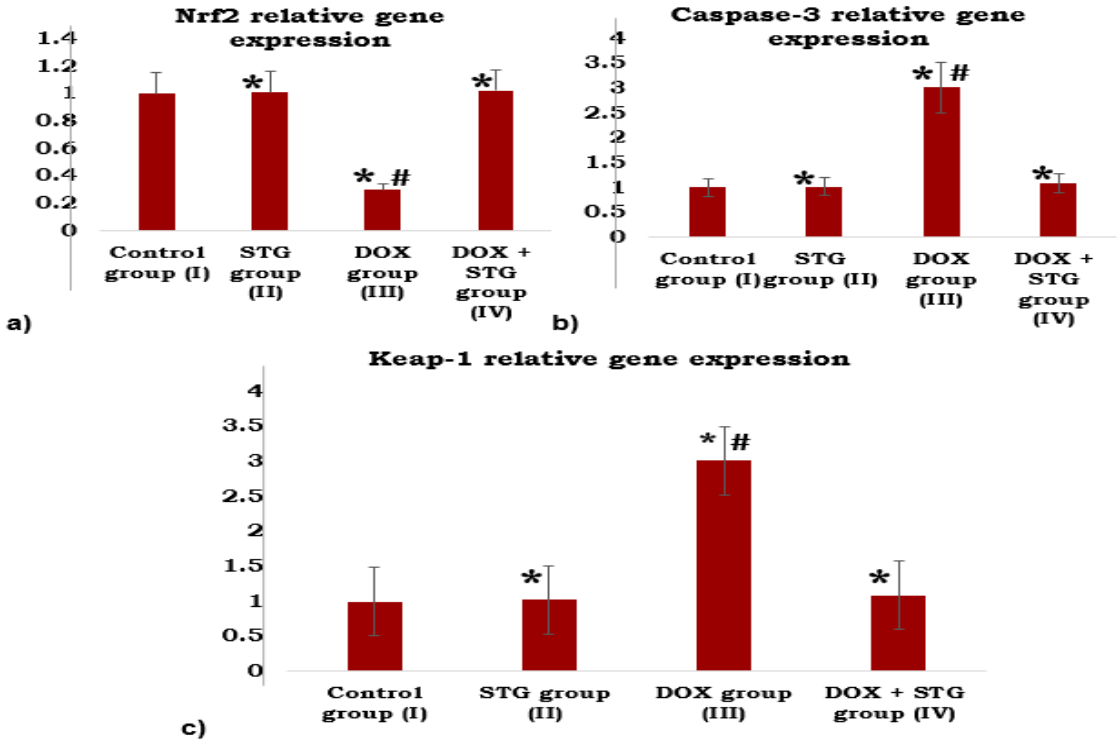
4.5. STG downregulates both cardiaccaspase-3, Keap-1 and upregulates NrF2 gene expression levels

The NrF2 gene expression level decreased in the STG group compared to the control group; however, this increase was not statistically significant. The treated group with STG showed a marked upregulation in NrF2 gene expression levels as compared to the DOX group, which was statistically significant (Fig4 a).

Regarding the caspase-3 gene expression level, the DOX group showed significant upregulation in caspase-3 gene expression as compared to the control group($p<0.05$). On the other hand, groups treated with STG showed a significant decrease in caspase-3 gene expression when compared to the DOX group ($p<0.05$) (Fig. 4b). DOX induction of cardiac toxicity significantly upregulated Keap-1 gene expression($p<0.05$) compared with the control group. However, treatment with STG significantly decreased keap-1 gene expression compared with the DOX group (Fig. 4c).

Nuclear factor erythroid 2-related factor 2, which has a regulating role in gene transcription in the cells, dramatically decreased in rats that received Dox compared to normal rats. STG had a protective role as Nrf2 was markedly increased in the DOX+STG group ($P<0.05$). The same observation was detected regarding caspase-3.

Figure 4: Evaluation of Nrf2 relative gene expression, Caspase-3 relative gene expression, Keap-1 relative gene expression.



DOX: Doxorubicin; STG: sitagliptin; Data are represented as Mean ± SD., n=8. P < 0.05 is significantly tested by ANOVA and post hoc multiple comparison LSD method. *: P < 0.05 vs. Control group; #: P < 0.05vs STG group, c: P < 0.05vs. DOX group

4.6. STG ameliorates DOX-induced cardiac histopathological changes

Cardiac sections from different groups were stained with H&E. The Control groupshowed normal cardiac muscle fiber with central round nuclei and normal striation (Fig.5 a &b). The DOX group showed irregular cardiac muscle fibers with multiple spaces between fibers containing extravasated blood cells and dilated blood vessels. The muscle fibers show vacuolated nuclei withinflammatory cellular infiltration and interstitial edema (Fig.5c&d). DOX+STG treated group showed regression of histopathological changes but with slightly thickened vessels (Fig. 5e&f). Mallory's trichrome stain sectioned from all groups was assessed as follows. In the control and STG groups, collagen fibers were seen mildly between muscle fibers (Fig. 6a & b). Conversely, the DOX-treated group revealed significant collagen fiber deposition between muscle fibers (Fig. 6c). The DOX + STG-treated group revealed minimal collagen fiber deposition between muscle fibers, resembling that of the control group (Fig. 6d).

Figure 5: A photomicrograph of a longitudinal section in the cardiac muscle in studied groups

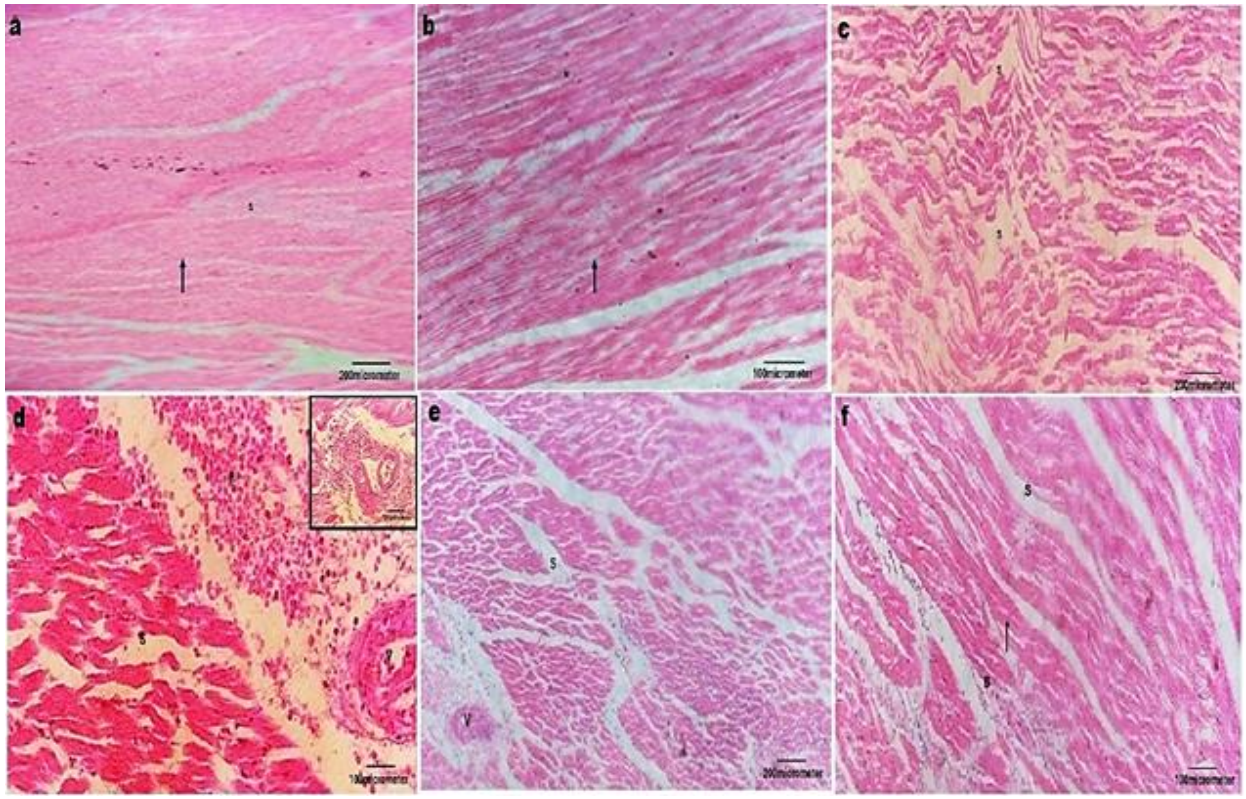


Figure 5: a). A photomicrograph of a longitudinal section in the cardiac muscle from the control group (group 1) reveals branched muscle fibers with nuclei positioned centrally (arrow) and acidophilic sarcoplasm (S). (H&E × 100). b) A photomicrograph of a longitudinal section in the cardiac muscle of STG-treated rats reveals longitudinally branched cardiac muscle fibers (arrow) and oval vesicular nuclei (N) are centrally located within cardiac muscle cells (H&E, ×200.c). A photomicrograph of a longitudinal section of cardiac muscle tissue from (the DOX-treated group) reveals degenerated and isolated cardiac muscle fibers, along with widened interfiber spaces (S). (H&E × 100). d) A photomicrograph of a longitudinal section of cardiac muscle tissue from (the DOX-treated group) reveals degenerated and isolated cardiac muscle fibers (S) showing blood leakage (indicated by arrows). Also notable are congested blood vessels (V) (H&E × 200). The inset showed extravasated blood between cardiac fibers (H&E × 200). e) A photomicrograph of a longitudinal section in the cardiac muscle from (the DOX+STG-treated group) showing, nearly similar to the control group, characterized by centrally located vesicular nuclei. However, wide interfiber spaces are still evident (S). with dilated blood vessels (V) (H&E X 400). f) A photomicrograph of a section in the cardiac muscle of DOX+ STG-treated rats, showing cardiac myocytes with normal architecture(arrow) and some separated cardiac muscle fibers (S). (H&E × 400).

Figure 6: A photomicrograph of the rat's cardiac muscle in studied groups

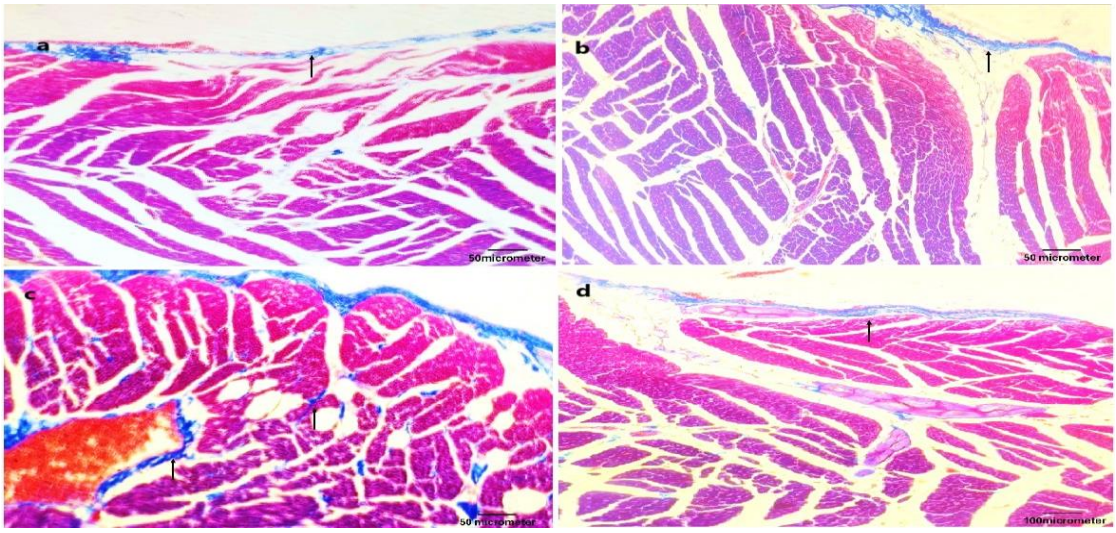


Figure. 6: a) A photomicrograph of the rat's cardiac muscle from the control group reveals mild collagen fiber deposition within the muscle fibers, the collagen fibers revealed a blue color (arrow). **b):** A photomicrograph of the rat's cardiac muscle from the STC-treated group reveals collagen fiber deposition within the muscle fibers(arrow). **c):** A photomicrograph of the rat's cardiac from the DOX-treated group reveals an increase in collagen fiber deposition within the muscle fibers and around blood vessels (arrow). **d):** A photomicrograph of the rat's cardiac STC + DOX-treated group reveals a decrease in collagen fiber deposition within the muscle fibers(arrow)**. (Mallory trichrome X 400).**

5. DISCUSSION

Doxorubicin (DOX) is a highly effective anticancer drug; however, its use is limited by its cardiotoxicity. Manifestations of its cardiotoxicity vary from arrhythmia, reduced ejection fraction, and cardiomyopathies to heart failure

The present study confirmed the cardiac damage associated with DOX administration. Increased oxidative stress was identified as the main mechanism of DOX-induced cardiotoxicity since several studies reported that DOX can generate ROS by multiple mechanisms in addition to producing downregulation of glutathione peroxidase **[15].**

Conventional antioxidants failed to prevent DOX-induced cardiac damage, so the search for additional mechanisms was mandatory. Since mitochondria are considered the main target organ for DOX-induced cardiotoxicity and the cardiac muscle is one of the tissues with the highest mitochondrial-containing tissues, the affection of this organelle will severely impair cardiac function. Multiple factors interact to cause DOX-induced mitochondrial dysfunction, such as impairing the structure and function of mitochondrial Creatine Kinase (CK), opening of mitochondrial permeability transition pores leading to apoptosis, reduction of the activity of cytochrome C oxidase, inhibiting members of sirtuin family, and disturbed mitochondrial iron homeostasis **[15].**

Nrf2 (nuclear factor erythroid-2 related factor 2) is a transcription factor that, when activated, regulates the expression of several genes responsible for antioxidant defense mechanisms, including the mitochondrial glutathione system, and thus the mitochondria become less vulnerable

to damage by ROS **[16].** The above fact was proven by the severe oxidative stress and apoptosis observed on deletion of Nrf2 in a model of diabetic bladder dysfunction in mice **[17].**

The pathogenesis of DOX-induced cardiotoxicity is closely linked to the inhibition of the Nrf2 signaling pathway, so targeting the activation of this pathway is mandatory in opposing its cardiotoxicity **[16].**

Nrf2 was found to inhibit many aspects related to cardiac damage, such as pathological remodeling and dysfunction, by reducing oxidative stress in several conditions. This occurs probably through a mediator role of Nrf2 in hydrogen sulfide-mediated suppression of oxidative stress. In addition, recently, Nrf2 was found to facilitate the autophagy of some toxic protein aggregates resulting from the accumulation of ROS, which reveals a novel protective mechanism of the Nrf2 signaling pathway **[17].**

Both the antioxidant and autophagic effects of Nrf2 activation make it a desirable target for the prevention or treatment of DOX-induced cardiotoxicity

On the other hand, iron accumulation is thought to potentiate DOX-induced cardiotoxicity, and DOX can trigger ferroptosis by Nrf2-mediated upregulation of heme-oxygenase1, which catalyzes heme degradation and accumulation of non-heme iron **[14].** Therefore, the activation of Nfr2 in DOX-induced cardiotoxicity can be a 2sided weapon one protective and the other potentiating, however, the present study revealed a marked reduction in the expression of Nrf2 concomitant with the administration of DOX and this agrees with a study that reported an exaggeration of DOX-induced cardiotoxicity in Nrf2 knockout mice **[16].**

Caspase 3 is a key mediator in apoptosis, which is activated in apoptotic cells by both death ligands and mitochondrial pathways but also shares in non-apoptotic functions such as tissue differentiation, regeneration, and neural development.DOX induces apoptosis through the activation of caspase-3, suggesting that apoptosis has an important role in the progression of DOX-induced cardiotoxicity **[18]**. Interestingly, this susceptibility to the apoptosis of cardiomyocytes under the effect of DOX decreased from neonatal to adult mice which means that it might be age dependent. In the present study, DOX-induced cardiotoxicity was associated with increased levels of caspase 3 apoptotic enzyme, and this was also confirmed by another study, which recommends that caspase 3 be a surrogate biomarker for DOX-induced cardiotoxicity **[19]**.

Numerous antioxidant pathways, including the Nrf2/Keap1signaling pathway, are involved in cellular redox homeostasis (4,27). Nrf2/Keap1 regulates the expression levels of antioxidants and phase II detoxification enzymes **[20]**.(39‑41). Nrf2is highly expressed in tissues where detoxification reactions take place and is a key regulator of the cellular defense mechanism in several organs, including the brain, lung, bladder, kidney, liver, and ovary, as well as macrophages and erythrocytes. The Nrf2/Keap1 signaling pathway maintains the healthy endothelial phenotype under normal physiological conditions. Endothelium is a type of tissue located at theborder between blood and tissues and is primarily involved inthe regulation of vascular

tone, thromboresistivity, inflammation of the vascular wall, and cellular adhesion **[21]**.IncreasedROS production in the endothelium activates Nrf2, whichcauses increased expression levels of intracellular HO-1, glutathione peroxidase (GPx), GSH, glutamate-cysteine ligasemodifier subunit, sulfiredoxin‑1, NQO1, protease‑activatedreceptor 4, and oxidative stress-induced growth inhibitor 1 genes in arterial endothelial cells. Studies using animal models have concluded that insufficient levels of Nrf2 in various types of tissue concurrently areassociated with susceptibility to several diseases, including cardiovascular disease **[22]**. Studies investigating therole of the Nrf2/Keap1 signaling pathway in atherosclerosis,hypertension, myocardial infarction and ischemia have beenconducted; however, to the best of our knowledge, there arecurrently no studies regarding Nrf2/Keap1 signaling in thromboembolism, which is the third leading cause of cardiovasculardisease-associated mortality, after coronary artery diseaseand stroke [19].In the present study, STG reduces both cardiac caspase-3,and Keap-1 upregulates NrF2 gene expression levels, and reduces oxidative stress.

The DPP-4 inhibitor, STG, is a widely used medication for the treatment of type 2 diabetes mellitus.Although clinical trials haven't exhibited a significant cardioprotective effect of DPP4 inhibitors and debate exists on its role in preventing heart failure, experimental studies have demonstrated beneficial effects on cardiac myocytes through improving mitochondrial function and inhibition of oxidative stress **[22],** which makes this drug a potential therapeutic agent against DOX-induced cardiac damage

These findings agreed with **El-Agamy, D. S., et al.,[23],** who reported that when rats were given STGbefore DOX administration, normal cardiac function was restored. However, a comparative study on whether pre/cotreatment of STG is more effective in reversing DIC is recommended.

STG, when combined with melatonin in diabetic cardiomyopathy, has been proven to improve mitochondrial biogenesis through the sirtuin1 mediated pathway **[24],** which was found to be suppressed by DOX

In another study, STG reduced oxidative stress and excessive autophagy through the p62-Keap1-Nrf2 signalling pathway and nuclear translocation of Nrf2 in a case of lung injury secondary to severe acute pancreatitis (20). Also, STG activated the Nrf2 pathway in a rat's brain-induced Alzheimer's disease **[25].**

Another study reported a downregulation of a KEAP 1 inhibitor, which is a negative regulator of Nrf2 activation by STG **[26].** All these studies agree with the results of the present study, which revealed an increase in Nrf2 expression on STG administration.

Overall, STG has been shown to have extra protective effects beyond glucose control. Studies indicate that it can reduce caspase-3 expression, thus potentially reducing cell death in various tissues. For example, antifibrotic and anti-apoptotic effects of STG were demonstrated in lung and testicular tissues influencing caspase activity **[27].** In the present study, STG reduced the levels of caspase 3 and prevented the activation

of NF-kappaB, proving its protective anti-apoptotic role in DOX-induced cardiotoxicity.

5. CONCLUSION:

In summary, DOX-induced cardiotoxicity is a fact with a multifactorial etiology of these excessive oxidative stress associated with the suppression of Nrf2 mediated antioxidant defense mechanisms and autophagy of toxic proteins, mitochondrial dysfunction, and increased apoptosis by activation of caspase 3 enzyme revealed. However, the cardiotoxicity is dose-dependent and sitagliptin administration -a DPP4 inhibitor- has shown a protective effect against DOX cardiac damage through decreasing apoptosis and activating the Nrf2 pathway combating the oxidative stress induced by DOX in the heart which makes a potential therapeutic agent against the harmful cardiac effects of DOX, however, the dose of sitagliptin and timing of administration (pre/co) and duration needs to be further investigated to reach optimum protection

6. CONFLICT OF INTEREST:

There is no conflict of interest

Author contributions:

M.M.H. and E.M.K. wrote the main manuscript text.W.H.M. and M.A.E. performed experiments and data analysis.A.Z.E. and M.K.A. assisted in the interpretation of results and manuscript revision. All authors reviewed and approved the final manuscript.

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