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Sitagliptin Mitigates Doxorubicin-Induced Cardiac Toxicity in Rats, Targeting TLR4/NF-Kb Signaling Pathway

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ABSTRACT

Background: The emergence of toxicities to vital organs, including the heart, has limited the use of the broad-spectrum antitumor drug doxorubicin (DOX). Sitagliptin (STG) offers a number of defensive advantages.

Objective: This study aimed to illustrate the possible underlying processes and cardioprotective impact of STG in cardiotoxicity caused by DOX.

Material and methods: DOX, DOX+STG, and control (10/group) were the three groups into which thirty male albino rats were divided. Cardiac index, serum cTnI, serum LDH, serum CK-MB, cardiac MDA, cardiac SOD, cardiac TNF-α, cardiac IL-6, cardiac IL-10, cardiac NF-kB gene expression and cardiac TLR4 gene expression. Furthermore, immunohistochemical and cardiac histology studies were carried out.

Results: There was dramatically elevated serum cTnI, serum LDH, serum CK-MB, cardiac MDA, cardiac TNF-α, cardiac IL-6 in addition to cardiac NF-kB and cardiac TLR4 immunoreaction and gene expression with substantial decline in cardiac index value, cardiac SOD and cardiac IL-10 of DOX group compared to control. STG dramatically ameliorated DOX induced cardiac changes.

Conclusion: STG offered cardioprotection in DOX induced cardiotoxicity by downregulating the cardiac TLR4/NF-kB pathway, anti-inflammatory and antioxidant functions.

Keywords: Doxorubicin, NF-kB, Cardiotoxicity, Sitaglibtin, TLR4.

INTRODUCTION

Significant adverse impacts that can prevent use of recently developed drugs, cause clinical studies to be stopped, and even lead to a drug's removal if it causes severe side effects as cardiotoxicity [(1)]. The majority of pharmacological classes may have cardiotoxic side impacts, but these impacts only manifest after long-term usage. For example, anticancer drugs may be a significant class of drugs that harm the heart either directly or through their metabolites [(2)].

Although doxorubicin (DOX), is highly efficient in treating a variety of cancers, its clinical applications may be limited if heart damage develops. Acute heart failure, cardiomyopathy, and cardiac arrhythmia are among DOX's cardiac toxicities [(3)]. The mechanisms of DOX-induced cardiotoxicity are explained by two primary theories: Iron-generated free radicals and mitochondrial dysfunction. DOX-induced cardiotoxicity is known to be attributed to increase in ROS production [(4)].

Cardiomyocytes express a number of Toll-like receptors (TLRs), including TLR4. During cardiomyopathy, these TLRs cause pathophysiological alterations in response to endogenous and external stimuli [(5)]. TLR4 signaling triggers the activation and production of NF-kB, which helps heart tissue generate pro-inflammatory cytokines [(6)]. The TLR, specifically TLR4 is linked to heart failure [(7)]. Therefore, one possible target for protection against DOX could be the suppression of the TLR4/ NF-κB pathway [(8)]. Since CVDs continue to be the largest cause of death despite indications of improvement in therapy, the primary focus in recent years has been on CVD prevention [(9)].

Sitagliptin are a widely used antihyperglycemic medication that works by raising GLP-1 levels [(10)]. Numerous clinical investigations have confirmed the medication's safety and effectiveness. It has also been demonstrated that sitagliptin (STG) is safe for the cardiovascular system [(11)]. Additionally, the medication was demonstrated to be successful in lowering the risk of CVS deterioration [(12)].

The first DPP4 inhibitor used to treat type 2 diabetes was sitagliptin. It is widely utilized as a monotherapy or as an adjuvant therapy to existing management [(13)]. It decreases NF-κB activation and inhibit the production of inflammatory mediators [(14)]. Additionally, it is well recognized that STG has anti-inflammatory and antioxidant qualities [(15)].

Through several different processes, STG protects the heart. It has been discovered that STG

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shields the heart from ROS. It plays a crucial part in suppressing damage caused by ROS and shields cells from the its harmful effects [16]. It's interesting that the majority of studies found that STG may have advantages unrelated to blood glucose regulation. This implies that a GLP-1R-independent pathway may be the primary mechanism by which DPP-4 inhibitors enhance cardiac function [17]. Therefore, investigating the cardioprotective impact of STG in DOX-induced cardiotoxicity and the possible underlying processes is what motivated us to carry out this investigation.

MATERIAL AND METHODS

Animals: The experiment was conducted using thirty mature male Wister albino rats weighing 140–190 grams after receiving the required permits from Faculty of Medicine's Research Ethical Committee with IRB NO: 3/2025ANAT17-1, Menoufia University, Egypt. ARRIVE criteria were followed throughout the experimental procedures. The rats were kept in cages with wire mesh (80 x 40 x 30 cm). Following two weeks of training under consistent environmental conditions.

Thirty rats were allocated equally into:

1- **Control group:** On days 8, 10, 12, 15, 17, and 19 after the trial began, rats were given an intraperitoneal (i.p.) injection of one milliliter of distilled water.

2- **DOX Group:** On days 8, 10, 12, 15, 17, and 19 after the trial began, the rats received i.p. injection of DOX (3 mg/kg) [18]. Egypt, HIKMA Specialized Pharmaceuticals provided doxorubicin in vials under the title "Adricin." There are 50 mg/25 ml of DOX HCL in each vial.

3- **Doxorubicin/STG-treated (DOX+STG) group:** On days 8, 10, 12, 15, 17, and 19 of the investigation, the rats received an intraperitoneal injection of DOX (3 mg/kg), and for three weeks starting on the first day of the trial, they received oral STG (10 mg/kg) dissolved in distilled water once daily [18].

Blood samples were obtained after three weeks of fasting. The rats' ultimate body weight was determined. The hearts were then removed, weighed, and prepared for histological and biochemical analysis using masson trichrom dye, H & E, and immunohistochemical evaluation.

The organ index was calculated by the following formula: Cardiac index = Organ weight/Bodyweight×100 [19].

Blood collection: All rats had blood samples taken from their retro-orbital plexus. At room temperature, the blood samples were left to coagulate for half an hour. Centrifugation was used to separate the serum for 15 minutes at 3000 revolutions per minute. Prior to the experiment, the serum was frozen at -20 degrees Celsius.

Utilizing the appropriate ELISA kits, the serum levels of cTnI, LDH, and CK-MB were assessed. Rat LDH ELISA Kit (Catalog No. MBS269777, MyBioSource Inc., San Diego, CA, USA), Rat cTnI ELISA Kit (Catalog Number: ab246529, Abcam, Cambridge, UK), and Rat CK-MB ELISA Kit (Catalog No. MBS2515061, MyBioSource Inc., San Diego, CA, USA).

Tissue homogenate preparation: A tissue homogenizer was used to homogenize the weighted cardiac tissues individually. The supernatant was collected and kept for the test at -80 °C.

Following the manufacturer's instructions, the ELISA Kit was used to quantify cardiac TNF-α (Cat.: MBS2507393, MyBioSource, Sandiego, CA, USA), cardiac IL-6 (Cat.: MBS269892, MyBioSource, Sandiego, CA, USA), and cardiac IL-10 (IL-10: ERI3010-1, Assaypro LLC, Saint Charles, Missouri, USA). In compliance with the manufacturer's instructions, cardiac MDA and SOD were measured using calorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt).

Quantitative assay of NF-kB and TLR4 genes expression using RT-PCR:

The QiagenRN easy plus Universal Kit from the USA was used to prepare cardiac tissues for total RNA isolation. The purity and quality of the RNA were then guaranteed. Until it was used, RNA was kept at -80 °C. Then, using an Applied Biosystems 2720 heat cycler (Singapore) for a single cycle, the first step involved synthesizing cDNA using the QuantiTect Reverse Transcription Kit from Qiagen in the USA. GAPDH primers served as an RNA loading control in RT-PCR assays. cDNA amplification was the second stage. SensiFASTTMSYBR Lo-ROX Kit, USA, employed cDNA in SYBR green-based quantitative real-time PCR for Relative Quantification (RQ) of NF-kB and TLR4 gene expression using the following primers that were created (Midland, Texas):

TCGACCTCCACCGGATCTTTC was the NF-kB forward primer, while
GAGCAGTCATGTCCTTGGGT was the reverse primer.
TCAGCTTTGGTCAGTTGGCT was the forward primer for TLR4, and
GTCCTTGACCCACTGCAAGA was the reverse.

Lastly, the Applied Biosystems 7500 software version 2.0.1 was used to analyze the data. The comparative ΔΔCt method, which normalizes the quantity of the target genes (NF-kB and TLR4) mRNA to GAPDH and compares it to a control, was used to perform the RQ of NF-kB and TLR4 gene expression.

Histopathological method:

For histopathological studies, Heart tissue sections were fixed at 10% neutral buffered formalin and put in paraffin. Sections were stained with H & E and Masson's Trichrome to detect the fibrosis.

Immunohistochemical studies:

The heart paraffin sections were incubated with hydrogen peroxide. Followed by blocking with BCA solution for 30 min and probed with primary antibody anti-TLR4 (1:100 dilution, (Servicebio, Wuhan, Hubei, China) and anti-NF-kB (monoclonal, dilution 1:200, Abcam). Finally, the intensities of TLR4 and NF-kB immune-stained fields were evaluated by the image J software (Maryland, USA) in 5 randomly selected different microscopic fields for each group.

Statistical analysis

The data was analyzed using SPSS version 16. The mean ± S.D was used to express the results. ANOVA and post hoc Tukey testing were used to assess the significance of group differences. P value ≤ 0.05 was deemed significant.

RESULTS

Serum cTnI, serum LDH, serum CK-MB, cardiac MDA, cardiac TNF-α, cardiac IL-6, cardiac NF-kB gene expression, and cardiac TLR4 gene expression were all markedly raised, while the DOX group's cardiac index value, cardiac SOD, and cardiac IL-10 were substantially lower than the control. Compared to the DOX, the DOX+STG had substantially higher cardiac index values, cardiac SOD, and cardiac IL-10, but significantly lower serum levels of cTnI, serum LDH, serum CK-MB, cardiac MDA, TNF-α, IL-6, cardiac NF-kB gene expression, and cardiac TLR4 gene expression (Table 1).

Table (1): The measured cardiac index, serum cTnI, serum LDH, serum CK-MB, cardiac MDA, cardiac SOD, cardiac TNF-α, cardiac IL-6, cardiac IL-10, cardiac NF-kB and TLR4 gene expression in all studied groups

	Control group	DOX group	DOX+STG group
Cardiac Index	0.71±0.06	0.48±0.03 *	0.58±0.04 *#
Serum cTnI (pg/mL)	21.18±0.9	32.2±1.32*	26.1±0.29 *#
Serum LDH (U/L)	32.5±1.82	80.28±3.1 *	51.26±3.17 *#
Serum CK-MB (pg/mL)	26.5±2.19	120.9±4.15 *	89.3±2.12 *#
Cardiac MDA (nmol/ gm. Tissue)	10.1 ±1.02	30.45± 2.12*	17.42± 0.92*#
Cardiac SOD (U/gm. Tissue)	4.99 ±0.09	2.45±0.11*	3.62±0.05*#
Cardiac TNF-α (pg/ml)	120.25±3.5	229.26±6.79*	172±3.1*#
Cardiac IL-6 (pg/mL)	181.25±7.89	290.8±6.15*	225±3.12*#
Cardiac IL-10 (ng/mL)	15.13±0.41	7.88±0.3*	11.7±0.22*#
Cardiac NF-kB gene expression	1	3.25±0.02*	2.44±0.04*#
Cardiac TLR4 gene expression	1	4.1±0.05*	3.41±0.07*#

* Significant compared with control, # Significant compared with DOX.

Histological results:

Histological sections of the control heart tissue stained with H & E revealed normal cardiac tissue architecture with myocardial cells organized normally. DOX displayed a hemorrhagic region and cardiac muscle fiber rupture. At ×400 magnification, Dox+STG displayed a minor hemorrhagic region (blue arrow) and an improvement in the architecture of the heart (black arrow) (Fig. 1: A-C).

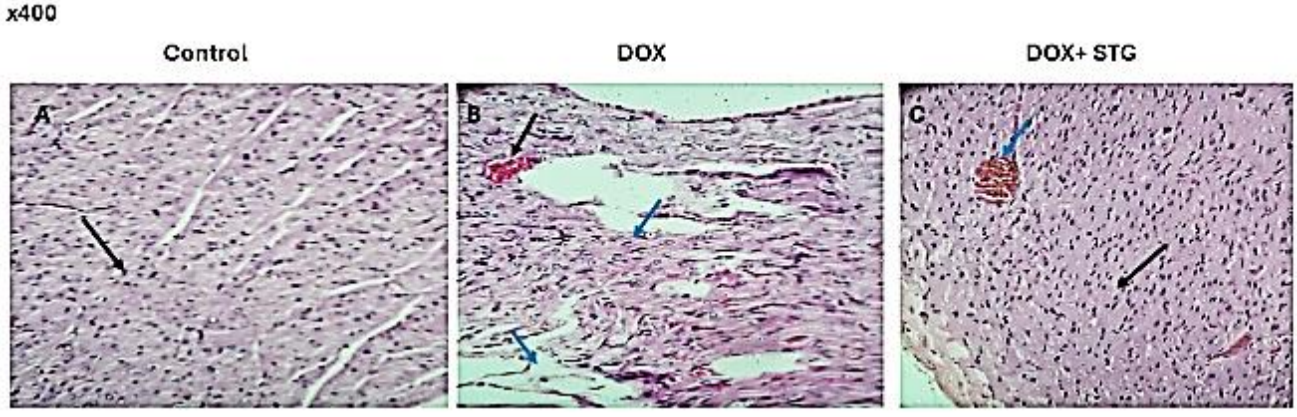

Figure (1): Normal cardiac tissue architecture with normally distributed myocardial cells was demonstrated by H & E sections of the heart of the (A) control group (arrow). (B) DOX displayed a hemorrhagic region (black arrow) and cardiac muscle fiber rupture (blue arrows). (C) DOX+STG at ×400 magnification, displayed a minor hemorrhagic region (blue arrow) and improved cardiac tissue architecture (black arrow).

When compared to control, the DOX's percentage area of Masson's Trichrome stained areas increased dramatically (48.6.6 ± 0.22 vs. 7.8 ± 0.15, respectively, $p < 0.05$). This proportion was elevated than that of the control group, but it was dramatically lower in DOX-STG than DOX (16.2.8 ± 0.45 vs. 48.6 ± 0.22, respectively, $p<0.05$) (Fig. 2: A-D).

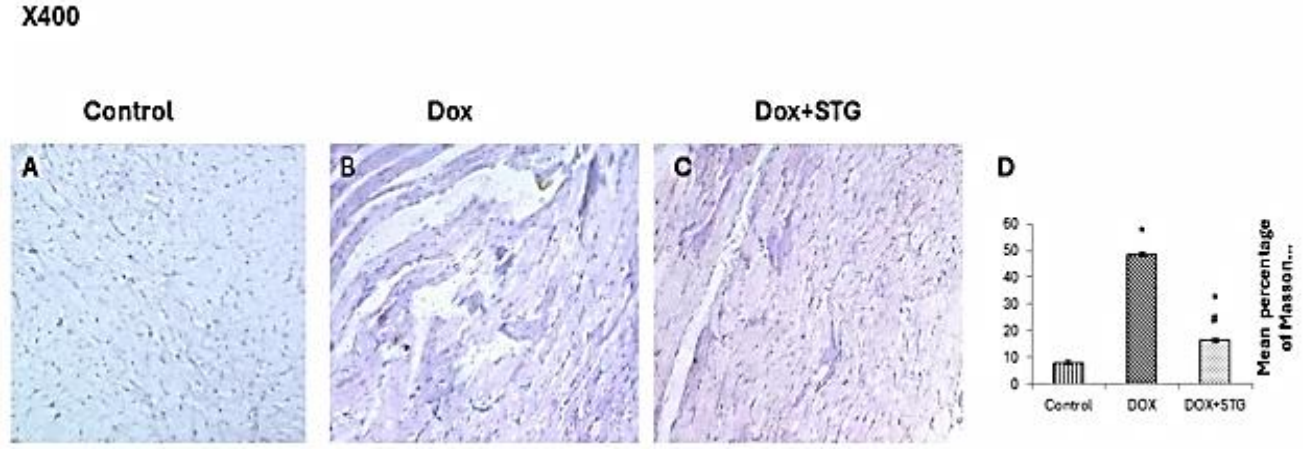

Figure (2): The Masson stain (A-D) was significantly downregulated in the DOX+STG and elevated in the DOX, according to representative micrographs of the various experimental groups.

Immunohistochemical results:

When compared to control, the DOX's percentage area of NF-kB was increased significantly (78.5 ± 0.23 vs. 9.8 ± 0.45, respectively, $p<0.05$) in the NF-kB stain. This proportion was elevated than that of the control, but it was dramatically decreased in the DOX+STG than DOX (29.4 ± 0.35 vs. 78.5 ± 0.23, $p<0.05$) (Fig. 3: A-D).

In the TLR4 stain, the DOX group's percentage area of TLR4 was dramatically higher than control (80.4 ± 0.41 vs. 10.5 ± 0.33, respectively, $p<0.05$). This proportion was higher than that of the control but it was dramatically lower in the DOX+STG than in the DOX (30.2 ± 0.05 vs. 80.4 ± 0.41., respectively, $p<0.05$). (Fig. 3: E-H).

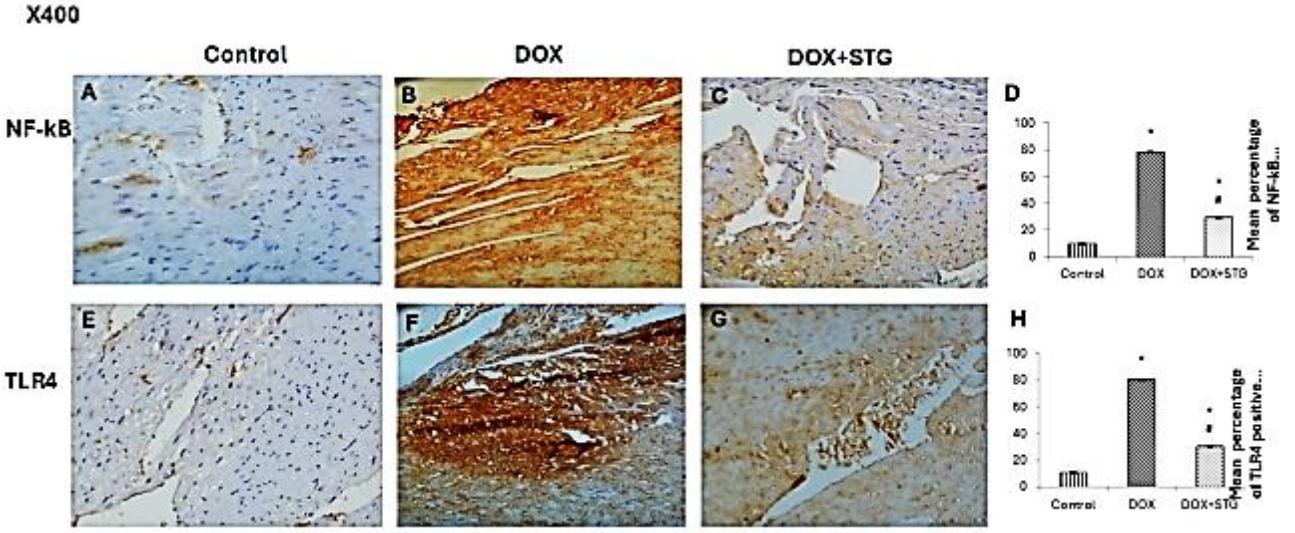

Figure (3): The NF-kB (A-D) and TLR4 (E-H) immunoreactions were dramatically increased in the DOX group and significantly downregulated in the DOX+STG group, according to representative micrographs of the various experimental groups.

DISCUSSION

Although DOX is chemotherapeutic medicines for cancer types, the danger of cardiomyopathy and congestive heart failure severely limits its clinical utility. By estimating a number of biochemical and histological markers, this study examined how STG affected DOX-induced cardiotoxicity. The findings demonstrated that STG had cardioprotective benefits against DOX cardiotoxicity due to its anti-inflammatory and anti-oxidative qualities.

Additionally, the marked increase in cardiac enzymes and decrease in cardiac index values and the rise in ROS after DOX administration, demonstrated the development of cardiac damage brought on by DOX administration. This may cause a number of biochemical markers, including CK-MB, which is regarded as a standard diagnostic metric for any type of

myocardial injury, to seep into the serum or plasma. This is consistent with earlier research [20], and histological analysis validated our findings.

Histopathological findings demonstrated that STG treatment reduced the production of these cardiotoxicity together with decreased cardiac enzymes into the serum. Combining these findings, the study indicates that STG has protective impacts against DOX, which is consistent with earlier research [18]. Furthermore, the impacts of STG on cytokine levels, NF-kB, and TLR4 were investigated. It is currently unknown how doxorubicin causes cardiotoxicity, however it is most likely a complex process. One of the hypothesized processes is cardiac muscle dysfunction, which can lead to heart failure. Another mechanism of DOX toxicity is oxidative stress; when free radicals damage the heart, membrane permeability rises, which promotes the release of cTnI, CK-MB, and LDH. DOX raised the levels of cTnI, CK-MB, and LDH in the current investigation. Troponin is typically released into the blood in larger quantities when the heart is damaged [18]. A high level of LDH, an enzyme that aids in energy production, is a biomarker for a number of illnesses, including cardiovascular disorders. It is found in the majority of body tissues, and its concentration rises during cell injury [21]. In line with earlier research, our findings showed that STG therapy greatly reduced the increase in cardiac enzymes [22].

Increased MDA and decreased SOD are indicators of DOX-induced oxidative stress. It is evident that oxidative damage, inflammatory response, and lipid peroxidation are some of the mechanisms underlying DOX toxicity. This aligns with earlier research [18].

The pathophysiology of DOX-induced cardiotoxicity involves several mechanisms. One of the main factors contributing to that is the overproduction of ROS and the reduction of endogenous antioxidants in the heart [23]. ROS causes oxidative damage to cellular components and lipid peroxidation, which results in the death of cardiomyocyte cells. This causes DOX to accumulate in the heart tissue, which is then more vulnerable to oxidative damage than other organs because there are fewer free-radical detoxifying enzymes and molecules, such as SOD [24].

The findings showed that DOX increased the amount of MDA in the heart, while significantly lowered SOD activity, which in turn promoted lipid peroxidation in the heart tissue. These results support numerous other investigations, which showed that the DOX-treated rats had worsening antioxidant state and elevated myocardial lipid peroxidation [24]. Because STG has a preventive effect against oxidative stress by reducing lipid peroxidation, its administration resulted in an amelioration of oxidative stress caused by DOX [25].

Additionally, STG can improve the antioxidant capacity. Since a GLP-1 receptor antagonist could counteract the antioxidant benefits of DPP-4 inhibitors, GLP-1 levels were raised. Furthermore, it has been demonstrated that GLP-1 activates AMP-activated protein kinase, which raises the activity of antioxidants and significantly reduces oxidative stress [26].

An important inflammatory sensor is the TLR-4/NF-kB pathway. By activating NF-κB and inducing pro-inflammatory cytokines, TLR-4 contributes to the control of inflammatory responses [27]. TLR-4 has been linked to DOX-induced cardiomyopathy, according to reports [28].

In line with earlier research, DOX administration in this study up-regulated TLR4 and NF-kB immunoreaction and gene expression in the DOX group's cardiac tissue relative to the control [29]. Furthermore, as demonstrated by the masson trichrom stain, DOX induced chronic inflammation by activating inflammation in comparison with the control. Cardiomyopathy was linked to continuous expression of several pro-inflammatory cytokines in heart tissue [30]. Increased ROS levels have the potential to dramatically raise TLR levels, particularly TLR4, and TLR4/NF-κB signaling, which in turn raises pro-inflammatory cytokine levels [31]. According to a prior study, TLR4 overexpression contributed to the etiology and development of heart failure, whereas TLR4-knockout animals given DOX showed no signs of this [7].

In cardiac tissue, STG significantly downregulated inflammation, TLR4, NF-kB immunoreaction and gene expression in comparison with the DOX group, which is consistent with earlier research [32]. We concluded that STG exhibited anti-inflammatory activity because of its potent suppression of TLR4 and NF-kB signaling cascade, and inhibition of the release of inflammatory cytokines, all of which may support STG's cardioprotective effects.

CONCLUSION

In DOX rats, STG offers cardioprotection by down-regulating the cardiac TLR4–NF-kB pathway, adjusting the heart's anti-inflammatory and antioxidant functions.

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