**The possible role of endothelial nitric oxide synthase (eNOS) in the effect of Sitagliptin on Doxorubicin induced cardiac dysfunction in adult male rats**

**By**

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**Abstract**:

**Background:** Cardiovascular diseases and cancer remain leading causes of mortality in the world. Doxorubicin (DOX) treatment is the primary cause of chemotherapy-induced cardiotoxicity. Sitagliptin (STG) has been shown to improve cardiovascular functions. Endothelial nitric oxide synthase (eNOS) is the major weapon of endothelial cells to fight vascular diseases as it generates nitric oxide (NO**). Aim:** The aim of this study was to evaluate the cardiotoxic effect of DOX on heart of adult male albino rats and the possible role of STG, targeting the role ofeNOS**. Methods:** The effect of DOX on albino rats was studied by estimating systolic blood pressure (SBP) , diastolic blood pressure (DBP) , E.C.G recording, Serum levels of creatine phosphokinase MB (CK-MB), lactate dehydrogenase (LDH), superoxide dismutase (SOD), malondialdehyde (MDA), cardiac tissue level of NO. **Results:**  DOX injection resulted in significant increases in both SBP and DBP, significant ST segment elevation and significant increases in serum levels of CK-MB, LDH and MDA associated with significant decreases in heart rate, cardiac NO levels and serum SOD levels as compared with the control group. STG administration reversed the aforementioned parameters. STG administration with NG-Nitro arginine methyl ester (L-NAME) and DOX reverse STG protective effects. **Conclusion:** STG administration can exert protective effects against doxorubicin induced cardiac toxicity through the increase in cardiac NO and e-NOS while its administration with L-NAME reverse these effects.

**Key words:** DOX, STG, eNOS, NO, L-NAME, SOD.

**Abbreviations:** CK-MB; **Creatine phosphokinase MB,** DBP; **diastolic blood pressure,** DOX; **doxorubicin,** eNOS; **Endothelial nitric oxide synthase**, LDH; **Lactate dehydrogenase**, MDA; **malondialdehyde**, NO; **nitric oxide**, SBP; **Systolic blood pressure,** SOD; [**superoxide dismutase**](http://www.scialert.net/asci/result.php?searchin=Keywords&cat=&ascicat=ALL&Submit=Search&keyword=superoxide+dismutase), STG; **Sitagliptin**, NG-Nitro arginine methyl ester **;(L-NAME).**

**1. INTRODUCTION:**

Doxorubicin (DOX) (also known as adriamycin) was isolated from the pigment-producing bacterium (Streptomyces peucetius var. caesius), and belongs to the family of anthracyclines (**1)**.

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 events at an early stage, and this risk remains high within 30 years after treatment **(2).**

The mechanisms to explain DIC are not completely understood. It seems to be a multistep process, with different potential pathways involved that leads to cardiomyocyte death **(3).** The main mechanisms that have been proposed by various research groups include oxidative stress, Ca2+ homeostasis dysregulation, sarcomeric structure alterations, gene expression modulation, and apoptosis **(4).**

STGis a highly selective DPP-4 inhibitor. Its major application is in the treatment of type 2 diabetes **(5).** STG exerts protective effects on the heart through multiple mechanisms. STG has been found protect the heart from ROS. It has an important role in the regulation of defensive genes activation and induction of antioxidant enzymes as SOD, catalase and glutathione peroxidase leading to suppression of injury evoked by ROS and protection of cells against oxidative stress injurious effects **(6).**

eNOS is the major isoform regulating vascular function. It is mostly expressed in endothelial cells. However, the isozyme has also been detected in cardiac myocytes, platelets, certain neurons of the brain, human placenta and kidney tubular epithelial cells **(7).**

NO has important autocrine/paracrine effects in the myocardium in physiological settings, in general serving to optimise and fine tune cardiac function. NO exerts small positive inotropic effects, which may serve to enhance basal cardiac function and enhances myocyte relaxation and diastolic function. NO also accelerates relaxation and reduces diastolic tone **(8)**.

This study was designed to demonstrate the cardiac effect of STG on DOX induced cardiotoxicity in adult male rats, targeting **the role of** eNOS.

**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)](https://www.medicines.org.uk/emc/product/7887/pil) (by Merck Sharp and Dohme Company, Northumberland, UK. Lot No: S008904) and **L-NAME,** provided as white powder (by Sigma, NewYork, U.S.A. Lot No: BCBK7552V).

**2.2. Animals:**

This experimental study was achieved using 24 adult male albino rats aged above 2 months and the weight is between 180 and 220 g. They were obtained from the animal research center, Faculty of Veterinary Medicine, Benha University. Each 4 rats were placed in a separate cage and were fed a standard diet, with free access to food and water. They were placed at room temperature (25o 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 complete healthy conditions all over the experiment and under care of a professional technician and a qualified researcher. All procedures were approved by ethical committee of Benha faculty of medicine. No rats were died throughout the experiment. At the end of the study the rats were incinerated at Benha university hospital incinerator.

**2.3. Experimental design:**

# The rats included in this study were classified into 4 main groups; Group (I) (Control group), rats were injected by saline intra peritoneal (i.p) (2.5 ml/kg/ day) for 3 weeks, Group (II) (DOX group): rats were injected by DOX (1mg/kg i.p per day) at rate of 5 injections per week, for 3 weeks to produce cumulative dose of 15mg/kg, Group (III) (STG group): same dose of [DOX](https://www.omicsonline.org/pharmaceutical-care-and-health-systems-open-access.php) was injected as in group II then rats were given STG (10 mg/kg/day) orally by esophageal tube for 3 weeks, Group (IV) ( STG +L-NAME group):DOX was injected i.p then rats were given STG (10 mg/kg/day) orally by esophageal tube then L-NAME was given in a dose of (40 mg/kg/day) orally by esophageal tube for 3 weeks.

**2.4. Procedure of the experiments:**

SBP, DBP and ECG of each rat were recorded at the end of the experiment, then the animals were fixed on operating table and a craniocaudal incision of about 2 cm was made, parallel with and slightly to the left of the sternum and the blood samples were taken from aorta then were put into non-heparinized tubes and allowed to clot. Then the hearts were excised, washed and stored.

**2.5. Parameters assay:**

**ABP measurement**

At the end of the experiment (on the 21st day of the experiment), ABP was measured by carotid artery cannulation through using Software Lab Chart 8 power lab recorder and analyzer (AD Instruments, Mountain View, CA, USA).

ECG study:

ECG was measured after ABP measurement for each rat by using Lab Chart 8 power lab recorder and analyzer (AD Instruments, Mountain View, CA, USA). Lead II was used in recording ECG, being the most informative one.

* **Blood sampling and preparation of serum:**

Serum was then separated from the clotted blood, by centrifugation at 5000 rpm for 10 min, and stored at -20°C till the assay of **(**CK-MB, LDH, SOD and MDA**)** levels.

* **Tissue sampling and preparation of heart homogenate:**

After collection of blood samples,light pressure on the thorax help to bring the heart outside without damage. The heart was divided longitudinally into two halves. Half was immediately washed with normal saline, kept in liquid nitrogen, and stored at -20° C for NO assays. The other half was kept in formaldehyde to be stained with Hematoxylin and Eosin (H&E).

**2.6. 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 one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test for comparison between every two groups. The p-values < 0.05 were considered statistically significant.

**3. RESULTS:**

**3.1. Effect of DOX on ECG and serum levels of (CK-MB and LDH): (Table 1):**

When rats were injected with DOX in DOX group at a dose 15mg/kg i.p for 3 weeks, there were significant increases (P < 0.05) in the ST segment amplitude (0.17 ±0.04) when compared with its corresponding in the control group (0 ± 0.00) and STG group (0 ± 0.00) while there was non- significant change (P > 0.05) in the ST segment amplitude (0.17 ±0.04) when compared with its corresponding in the STG + L-NAME group (0.16 ± 0.06 ).

There were significant increases in the serum CK-MB level (602.83 ± 37.15) in the DOX group when compared with its corresponding in the control group (314.67 ± 35.16) and STG group (317 ± 31.16 ) while there was non-significant change in serum CK-MB level (602.83 ± 37.15) when compared with its corresponding in the STG + L-NAME group (610.33 ± 32.46).

Moreover, there were significant increases in the serum LDH level (318 ± 26.75) in the DOX group when compared with its corresponding in the control group (197 ± 15.28) and STG group (201.17 ± 18.50) while there was non-significant change in serum LDH level (318 ± 26.75) when compared with its corresponding in the STG + L-NAME group (332.17 ± 37.51).

**3.2. Effect of DOX on serum level of SOD, MDA and cardiac level of NO (Table 1):**

There were significant decreases in the serum SOD level (6.32 ± 1.47) in the DOX group when compared with its corresponding in the control group (14.13 ± 2.97) and STG group (19.70 ± 1.84) while there was non-significant change in serum SOD level (6.32 ± 1.47) when compared with its corresponding in the STG + L-NAME group (5.57 ± 1.29).

Moreover, there were significant increases in the serum MDA level (3.21 ± 0.60) in the DOX group when compared with its corresponding in the control group (1.90 ± 0.40) and STG group (1.26 ± 0.24) while there was non-significant change in serum MDA level (3.21 ± 0.60) when compared with its corresponding in the STG + L-NAME group (2.73 ± 0.32).

In addition, there were significant decreases in the cardiac NO level (6.27 ± 1.24) in the DOX group when compared with its corresponding in the control group (12.40 ± 1.74) and STG group (20.14 ± 2.23) while there was non-significant change in the cardiac NO level (6.27 ± 1.24) when compared with its corresponding in the STG + L-NAME group (6.24 ± 1.49).

**3.3. Effect of DOX on SBP and DBP (Table 1):**

There were significant decreases in the SBP level (82.17 ± 5.49) in the DOX group when compared with its corresponding in the control group (116.83 ± 4.71) and STG group (118 ± 5.10) while there was non-significant change in SBP level (82.17 ± 5.49) when compared with its corresponding in the STG + L-NAME group (85.33 ± 6.80).

Moreover, there were significant decreases in the DBP level (45.67 ± 6.77) in the DOX group when compared with its corresponding in the control group (77.83 ± 4.53) and STG group (78.67 ± 2.50 ) while there was non-significant change in DBP level (45.67 ± 6.77) when compared with its corresponding in the STG + L-NAME group (43 ± 5.80).

Table 1: Comparison of ECG changes{ ST segment amplitude (m.v), heart rate (cycle*/* min)}, levels of serum CK-MB (U*/*L), serum LDH(μ/U), serum SOD (U/mL), serum MDA (nmoL/mL), cardiac NO (μmol/g), SBP (mmHg) and DBP (mmHg) between control, DOX, STG and STG+ L-NAME groups

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Groups** | **Control group (I)** | **DOX group****(II)** | **STG group****(III)** | **STG +****L-NAME group (IV)** | **P Value** |
| **ST segment amplitude (m.v)** | 0 ± 0.00 | 0.17 ±0.04a,c | 0±0.00b,d | 0.16 ± 0.06a,c | < 0.05 |
| **Heart rate****(cycle/min)** | 356.67 ± 39.20 | 205 ± 23.24a,c | 368.33 ± 43.55b,d | 206.67 ± 21.13a,c | < 0.05 |
| **Serum CK-MB(U/L)** | 314.67 ± 35.16 | 602.83 ± 37.15a,c | 317 ± 31.16b,d | 610.33 ± 32.46a,c | < 0.05 |
| **Serum LDH (μ/U)** | 197 ± 15.28 | 318 ± 26.75a,c | 201.17 ± 18.50b,d | 332.17 ± 37.51a,c | < 0.05 |
| **Serum SOD (U/mL)** | 14.13 ± 2.97 | 6.32 ± 1.47a,c | 19.70 ± 1.84a,b,d | 5.57 ± 1.29a,c | < 0.05 |
| **Serum MDA (nmoL/mL)** | 1.90 ± 0.40 | 3.21 ± 0.60a,c | 1.26 ± 0.24a,b,d | 2.73 ± 0.32a,c | < 0.05 |
| **CardiacNO (μmol/g)** | 12.40 ± 1.74 | 6.27 ± 1.24a,c | 20.14 ± 2.23a,b,d | 6.24 ± 1.49a,c | < 0.05 |
| **SBP (mmHg)** | 116.83 ± 4.71 | 82.17 ± 5.49a,c | 118 ± 5.10b,d | 85.33 ± 6.80a,c | < 0.05 |
| **DBP (mmHg)** | 77.83 ± 4.53 | 45.67 ± 6.77a,c | 78.67 ± 2.50b,d | 43 ± 5.80a,c | < 0.05 |

Data are represented as Mean ± SD:

asig from Group I - bsig from Group II - csig from Group III - dsig from Group IV

- P value < 0.05 was considered statistically significant

- P value > 0.05 was considered statistically insignificant

ST segment amplitude (m.v), heart rate (cycle*/* min)}, levels of serum CK-MB (U*/*L), serum LDH(μ/U), serum SOD (U/mL), serum MDA (nmoL/mL), cardiac NO (μmol/g), SBP (mmHg) and DBP (mmHg).

**4. DISCUSSION:**

In spite of the accumulating data about doxorubicin induced cardiac toxicity (DIC) the prophylactic role of STG in the DIC has to be established. Hence, this study was designed to detect the effect of STG on a rat model of DIC targeting the role of eNOS.

The data of the present work revealed that, with respect to the control group, DOX injection yield to significant changes in the ECG presented by elevated ST segment in addition to significant decrease in heart rate (P<0.05) with significant increases in serum CK-MB and LDH levels (P<0.05) as seen in (Table 1). This was in agreement with **(9)** who reported that DIC was attributed to elevate ST segment, decrease heart rate and increase in serum CK-MB and LDH levels. Moreover, it decreases O2 saturationleading to hypoxia, ischemia and necrosis of myocardium with releasing of cardiac myocytes intracellular content to the extracellular fluids including CK-MB and LDH. Collectively, all these factors led to profound ECG changes and rise of serum CK-MB and LDH levels and extensive cardiac injury.

The DPP-4 inhibitor, STG, is a widely used medication for treatment of type 2 diabetes mellitus **(10).** However, its prophylactic role against DIC is still a matter of debate. When STG was supplemented with DOX for 3 weeks in our study, it had restored the normal ECG pattern with significant increases in heart rate up to the control values (368.33 ± 43.55) when compared to the DOX group (205 ± 23.24) as and significant decreases in serum CK-MB and LDH levels (317 ± 31.16) and (201.17 ± 18.50) respectively when compared to the DOX group (602.83 ± 37.15) and (318 ± 26.75) respectively. These findings were in agreement with **(11)** who reported that when rats were given STG before DOX administration, this leads to restoring normal cardiac function.

To investigate the mechanism by which STG ameliorates the manifestations of DIC, the cardiac tissue level of NO was measured. Our study revealed that the DOX group exhibited significant decreases in the cardiac levels of NO (6.27 ± 1.24), when compared to control group (12.40 ± 1.74) as (P<0.05) as seen in (Table 1).This finding was in agreement with **(12)** whoreported that when mice were injected with DOX leads to significant decrease in NO level. This could be explained by DOX treatment induces endothelial toxicity which has been linked to excessive ROS generation, this leads to increases intracellular oxidant stress, disruption of NO/superoxide balance, decreases of NO and alteration the endothelial elasticity. In other words, DOX reduces eNOS which leads to decrease the bioavailability of NO in endothelial cells which contributes to endothelial dysfunction **(13).**

Furthermore, STG group exhibited significant increases in cardiac NO levels (20.14 ± 2.23) when compared to DOX group (6.27 ± 1.24) as (P< 0.05) as seen in (Table 1). These findings revealed the antioxidant properties of STG administration. In supporting of our findings**, (11)** found that when rats were given STG at a dose of 20 mg/kg, orally for 10 days before DOX administration, while DOX was given one dose on the 10th day in a dose of 20 mg/kg, i.p this leads to an increase in cardiac NO level. Also, **(14)** found that when diabetic rats were given STG therapy leads to an increase in cardiac NO. This could be explained by activation of GLP-1 with STG causes up regulation in intracellular cascades like protein kinases and cGMP, mediated NOS upregulation and e-NOS production **(15).**

Our study also investigated the serum level of antioxidant enzyme SOD and MDA as a marker for lipid peroxidation. The DOX group exhibited significant increase in serum level of MDA (3.21 ± 0.60) that was coupled by significant decrease in the serum level of SOD (6.32 ± 1.47) when compared to control group (1.90 ± 0.40) and (14.13 ± 2.97) respectively as (P<0.05) as seen in (Table 1). Our results were in agreement with **(16)** who found that DOX injection leads to increase serum MDA level and decrease serum SOD level**.** These findings indicate that DIC is associated with oxygen species that stems from an imbalance between oxidative radicals and anti-oxidative enzymes **(17).**

Regarding to STG group, there was significant decrease in serum level of MDA (1.26 ± 0.24) that was coupled by significant increase in the cardiac level of SOD (19.70 ± 1.84) when compared to the DOX group (3.21 ± 0.60) and (6.32 ± 1.47) respectively as seen in (Table 1).These findings revealed the antioxidant properties of STG. In supporting of our findings, **(18)** who revealed that STG activates redox-sensitive transcription factors leading to enhancing the expression and activities of SOD while down regulating NADPH oxidase so, maintaining the membrane integrity and restricting the leakage of CK-MB enzyme hence lowering activities of CK-MB and LDH enzyme in the serum. Noteworthy, there were a non-significant changes in all parameters between STG group and control group except in cardiac level of NO which had significant increase in the STG group (20.14 ± 2.23) when compared to the control group (12.40 ± 1.74) as (P<0.05) as seen in (Table 1) denoting the powerful antioxidant properties of STG.

Furthermore, our current study indicated that DIC was associated with hypotension. This was evidenced by the significant decreases in SBP and DBP levels in the DOX group (82.17 ± 5.49) and (45.67 ± 6.77) respectively when compared to control group (116.83 ± 4.71) and (77.83 ± 4.53) respectively as seen in (Table 1). With supporting of our finding, **(19)** whohad reported that DOX resulted in decrease in both SBP and DBP. This could be explained by that chronic administration of DOX leads to both systolic and diastolic heart failure decreasing both SBP and DBP **(20).** DIC also leads to increase in iNOS levels which is responsible for the production of shock-inducing NO that leads to sever vasodilatation and hypotension **(21).**

In addition, STG administration with a dose of 10 mg/kg/ day for 3weeks with DOX at a dose of 15 mg/kg i.p for 3 weeks led to an improvement in ABP. This was evidenced by the significant increase in SBP and DBP up to normal in STG group (118 ± 5.10) and (78.67 ± 2.50) respectively when compared with DOX group (82.17 ± 5.49) and (45.67 ± 6.77) respectively as (P <0.05) as seen in (Table 1). These data were consistent with **(22)** who had found that when rats were pretreated with STG leads to elevated blood pressure levels of endotoxemic rats as lipopolysaccharide injection induced a severe septic shock condition with hypotensive blood pressure levels. STG exerts its normotensive effects by decreasing iNOS activity (**22)** which extremely increases in pathological conditions like DIC. STG also has anti-hypertensive effects mainly relying on improving endothelial functions as it decreases endothelium dependent contractions to relieve hypertension related vascular events, decreases urinary angiotensinogen and renal cortical concentration of angiotensin II (**23)**.

Finally, in the present study when STG was supplemented with L-NAME and DOX in STG+ L-NAME group this resulted in significant increases in serum CK-MB and LDH levels (610.33 ± 32.46) and (332.17 ± 37.51) respectively with significant decrease in cardiac level of NO and serum level of SOD (6.24 ± 1.49) and (5.57 ± 1.29) respectively that was coupled by significant increase in the serum level of MDA (2.73 ± 0.32) when compared to the STG group (20.14 ± 2.23) & (19.70 ± 1.84) &(1.26 ± 0.24) respectively and control group (12.40 ± 1.74)& (14.13 ± 2.97 ) &(1.90 ± 0.40) respectively.

Moreover, there was significant decreases in SBP and DBP levels in the STG+ L-NAME group (85.33 ± 6.80) and (43 ± 5.80) respectively when compared to STG group (118 ± 5.10) & (78.67 ± 2.50) respectively and control group (116.83 ± 4.71) & (77.83 ± 4.53) respectively. This can be explained by L-NAME is a non-selective NOS inhibitor and inhibits the activities of the three NOS isoforms: e-NOS, inducible NOS (i-NOS) and neuronal NOS (n-NOS) **(24),** so L-NAME decreases NO and inhibit STG protective cardiac effects.

**5. CONCLUSION:**

From the current study, we can conclude that STG administration can exert protective effects against DIC through increasing cardiac NO and e-NOS while its administration with L-NAME reverse these effects.

**6. CONFLICT OF INTEREST:**

There is no conflict of interest

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