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Protective Role of Quercetin in Amelioration of Nitroglycerin-induced Nitrate Tolerance in Albino Rats

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Abstract

Background: Organic nitrates are extensively utilised for managing angina pectoris. However, prolonged administration of Nitroglycerin (GTN) results in tolerance, a phenomenon associated with heightened Reactive Oxygen Species (ROS) production. Quercetin, a potent natural antioxidant, exhibits the capability to neutralise oxidative free radicals, thereby potentially mitigating the onset of nitroglycerin tolerance. **Aim:** To estimate the protective effect of quercetin against GTN-induced nitrate tolerance in albino Wistar rats by evaluating oxidative stress and antioxidant biomarkers. **Methods:** Tolerance was induced in albino Wistar rats through daily administration of GTN (30 mg/kg). Vascular functionality was assessed by measuring Superoxide Dismutase (SOD), nitrates, Malondialdehyde (MDA), Reduced Glutathione (GSH), γ-Glutamyl transferase (γ-GluT), and total protein concentrations in both blood samples and isolated heart preparations. **Results:** Chronic GTN administration led to impaired vascular responsiveness and a substantial escalation in ROS production. However, concurrent quercetin administration significantly reduced GTN-induced oxidative stress. **Conclusion:** Prolonged GTN infusion enhances mitochondrial ROS generation, serving as a pivotal mechanism in the development of nitrate tolerance and cross-tolerance. Quercetin appears to attenuate nitrate tolerance by counteracting oxidative stress.

Major Findings: Chronic GTN administration induced oxidative stress and changed antioxidant status in albino Wistar rats. Quercetin treatment, mainly at 20 mg/kg, suggestively restored antioxidant defence and minimised oxidative damage, illustrating its protective effect against GTN-induced nitrate tolerance.

Keywords: Endothelial Cells, Nitrate Tolerance, Reactive Oxygen Species, Vasodilators

Abbreviations: ANOVA: Analysis of Variance; cGMP: Cyclic guanosine monophosphate; DMSO: Dimethyl sulfoxide; EEL: External elastic lamina; eNOS: Endothelial nitric oxide synthase; GGT: Gamma-glutamyl transferase; GSH: Reduced glutathione; GTN: Glyceryl trinitrate (Nitroglycerin); GTP: Guanosine triphosphate; H and E: Hematoxylin and eosin; IAEC: Institutional Animal Ethics Committee; IEL: Internal elastic lamina; i.p.: Intraperitoneal; ISDN: Isosorbide dinitrate; ISMN: Isosorbide-5-mononitrate; MDA: Malondialdehyde; NADPH: Nicotinamide adenine dinucleotide phosphate; NO: Nitric oxide; PBS: Phosphate-buffered saline; pVASP: Vasodilator-stimulated phosphoprotein; ROS: Reactive oxygen species; SEM: Standard Error of the Mean; sGC: Soluble guanylate cyclase; SOD: Superoxide dismutase; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid; TNB: 5-Thio-2-nitrobenzoic acid; TPC: Total protein concentration; VVG: Verhoeff–Van Gieson.

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1. Introduction

Organic nitrates rank among the most extensively utilised pharmacotherapeutic agents for the management of angina pectoris[1,2]. They are frequently utilised in the treatment of congestive heart failure. Despite their therapeutic efficacy, the precise mechanisms underlying nitrate bioactivation and the development of nitrate tolerance remain incompletely elucidated and are likely multifaceted[3,4]. Organic nitrates function as prodrugs that undergo bioconversion within vascular smooth muscle cells, yielding Nitric Oxide (NO) as their active metabolite[4,5]. NO subsequently activates Soluble Guanylate Cyclase (sGC), an intracellular enzyme that catalyses the conversion of Guanosine Triphosphate (GTP) into cyclic Guanosine Monophosphate (cGMP), a pivotal secondary messenger responsible for mediating vasodilation and other cardiovascular effects[6]. Elevated intracellular cGMP levels promote the sequestration of calcium ions into the sarcoplasmic reticulum, thereby reducing cytosolic calcium concentrations and facilitating vascular smooth muscle relaxation[7].

The most commonly used nitrates include GTN, Isosorbide Dinitrate (ISDN), and Isosorbide-5-Mononitrate (ISMN). While these agents exhibit analogous mechanisms of action, their therapeutic benefits are mediated through NO-dependent vasodilation.

2. Materials and Methods

2.1 Materials

For *in vivo* induction of nitrate tolerance, GTN was procured as an injectable solution from Chandra Bhagat Pharma Pvt. Ltd. (Palghar, Maharashtra). Quercetin was sourced from SD Fine Chem. Ltd. (Mumbai). All other reagents utilised were of analytical grade and obtained from Sigma-Aldrich, Loba, SDS, and Kemphaol.

2.2 Animal Model and *In Vivo* Treatment

All experimental procedures were conducted in accordance with the institutional animal ethics guidelines with an approval number of 009/IAEC/NCPA/M.Pharm/2012-2013. Albino Wistar rats were maintained at a 12-h light-dark cycle, 23±2°C temperature, and 45-55% humidity. The animals were acclimated to the laboratory atmosphere before starting the experiment. Thirty-six Wistar rats (6–8 months old) were randomly assigned to six groups (n=6 each): Control groups received water for injection. The Vehicle Control group received water for injection (i.p.). GTN-Induced Nitrate Tolerance: Four groups received GTN 30 mg/kg/day, i.p., in three doses over 8 days)[4]. Quercetin + GTN: Two groups received GTN with quercetin (10 mg/kg/day or 20 mg/kg/day). Quercetin Control was given with 20 mg/kg/day quercetin, i.p., dissolved in Dimethyl Sulfoxide (DMSO)[8]. GTN + Vitamin E: GTN was given with Vitamin E (10 mg/kg/day) as an antioxidant comparator[9]. After treatment, blood samples and heart homogenates were collected for biochemical analysis, and the animals were euthanised. Vitamin E supplementation has been shown to inhibit the depletion of Vasodilator-Stimulated Phosphoprotein (pVASP), a marker of NO/cGMP pathway activity, thereby improving vascular responsiveness. The data are expressed as mean ± SEM (Standard Error of the Mean).

2.3 Heart Homogenate Preparation

Fresh heart tissues were rapidly excised from Wistar rats following euthanasia and immediately rinsed in ice-cold Phosphate-Buffered Saline (PBS, pH 7.4) to remove residual blood. The tissues were then blotted dry and weighed. Each heart was minced into small fragments using sterile surgical scissors on a chilled glass plate to prevent enzymatic degradation.

Homogenisation was carried out in ice-cold homogenisation buffer composed of 50mM Tris-HCl (pH 7.4), 150mM NaCl, 1mM EDTA. The minced heart tissue was homogenised using a motorised tissue homogeniser with approximately 10–15 strokes on ice to preserve protein and enzymatic activity. The resulting homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C. The supernatant was carefully collected for subsequent biochemical analyses[10].

2.4 Collection of Serum Sample

Blood samples were collected from rats under light anaesthesia to minimise distress[11]. Collection was performed via retro-orbital plexus puncture. Approximately 1–2mL of blood was drawn using a sterile syringe and immediately transferred into plain

glass tubes without anticoagulant to allow for serum separation.

The blood was allowed to clot undisturbed at room temperature for 30 minutes, after which it was centrifuged at 3000 rpm for 15 minutes at 4°C. The clear supernatant (serum) was carefully aspirated using a micropipette and transferred into sterile tubes. All procedures adhered strictly to institutional animal ethics guidelines and were approved by the Institutional Animal Ethics Committee (IAEC).

3. Biochemical Analyses

3.1 Estimation of Superoxide Dismutase (SOD) Activity[10]

SOD catalyses the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen. The availability of superoxide dismutase, an enzyme that removes superoxide radicals by catalysing their dismutation into O_2 and H_2O_2, provided an opportunity to investigate the involvement of O_2^-. Heart homogenates were prepared using sodium phosphate buffer (0.01M). The reaction mixture contained 0.88mL of riboflavin (1.3×10^{-5} M in 0.01M potassium phosphate buffer), 60µL of O-dianisidine solution (0.01M ethanol), and 100µL of the sample containing SOD. Absorbance was measured at 460nm, and a standard curve was plotted using bovine SOD.

3.2 Lipid Peroxidation Assay (Malondialdehyde, MDA Estimation)[12]

Free radicals (ROS) attack membrane lipids, causing lipid peroxidation and forming MDA as a secondary product. MDA reacts with two molecules of Thiobarbituric Acid (TBA) under acidic and high-temperature conditions. The reaction produces a TBA-MDA adduct that has a pink colour. A 0.5mL sample was mixed with 0.5mL of 30% TBA and vortexed at 3000 rpm for 30 seconds. The supernatant (500µL) was combined with an equal volume of deionised water and heated at 98°C for 1 hour. The reaction was cooled on ice for 5 minutes, and the resulting pink-colored complex was analysed spectrophotometrically at 532nm. Absorbance values were compared against a standard curve of 1,1,3,3-tetraethoxypropane.

3.3 Estimation of Total Protein Content[13]

Proteins contain peptide bonds (-CO-NH-), and in alkaline conditions, these peptide bonds react with copper (II) ions (Cu^{2+}) to form a violet-colored complex. The intensity of the violet colour is directly proportional to the protein concentration. The absorbance is measured spectrophotometrically, usually at 540–560nm. Three sets of samples (blank, standard, and test) were prepared using Biuret reagent (1mL) and either a blank solution, a protein standard, or the test sample (0.02mL). The intensity of the colour developed was analysed using a bioanalyser.

3.4 Determination of γ-Glutamyl Transferase (γ-GluT) Activity[14]

The principle involved in the determination of γ-Glutamyl Transferase (Γ-GLUT) activity is based on an enzymatic reaction where Γ-GLUT catalyses the transfer of the γ-glutamyl group from a donor substrate to an acceptor. γ-Glutamyl Transferase (Γ-GLUT) catalyses the transfer of the γ-glutamyl group from L-γ-glutamyl-4-nitroanilide (the substrate) to an acceptor such as glycylglycine, forming γ-glutamyl-glycylglycine and 4-nitroaniline, which is chromogenic (yellow-colored compound). The working reagent was prepared by dissolving the substrate (gamma-glutamyl para-nitroanilide) in 2.2mL of buffer reagent (0.04M L-serine and 0.04M sodium borate; pH 8). The mixture was incubated at 37°C for 1 minute, followed by the addition of 0.1mL of the sample containing Γ-GLUT. Absorbance (A_0) was recorded at 1-minute intervals for up to 3 minutes. The mean change in absorbance per minute was calculated.

3.5 Determination of Nitrite Concentration (Griess Method)[15]

The principle of the reaction is that the nitrate present in the sample is reduced by the cadmium column to NO2-, which reacts with a Griess reagent to form a purple azo dye. Nitrite in a sample does not require reduction and reacts with the Griess reagent whether the cadmium column is bypassed or utilised[15].

The Griess reagent is composed of one part 0.1% naphthylethylenediamine dihydrochloride in distilled water and one part 1% sulfanilamide (or sulfanilic acid) in 5% concentrated H_3PO_4. The two parts are mixed within 12 hours of use and kept chilled[15]. A reaction

mixture comprising 100µL of Griess reagent and 300µL of the nitrate-containing sample was prepared, followed by the addition of 2.6mL of deionised water. The mixture was incubated at room temperature for 30 minutes, and optical density was recorded at 546nm. A standard curve was constructed using Sodium Nitrate ($NaNO_3$) with concentrations ranging from 1–100µM[16].

3.6 Quantification of Reduced Glutathione (GSH) Levels[17]

GSH contains a free sulfhydryl (-SH) group. When 5,5'- dithiobis-(2- nitrobenzoic acid) (DTNB) is added to a solution containing GSH, it reacts with the -SH group to form a yellow-coloured product: 5-thio-2-nitrobenzoic acid (TNB). The intensity of the yellow colour formed is directly proportional to the concentration of GSH. A 0.5mL sample was mixed with 0.5mL of 5% Trichloroacetic Acid (TCA) and centrifuged for 20 minutes to precipitate proteins. The supernatant (0.1mL) was mixed with 1mL of Disodium Hydrogen Phosphate (Na_2HPO_4) buffer and 0.5mL of DTNB, forming a yellow-coloured complex. Optical density was recorded at 412nm, and a standard curve was plotted using purified glutathione.

4. Results

4.1 Concentration of Biochemical Parameters in Serum and Heart Homogenate Samples

4.1.1 Concentration of SOD in Serum and Heart Homogenate Samples of Different Treated Animals

GTN-treated rats showed a 22% decrease in serum SOD levels compared to controls. Pre-treatment with quercetin (10 and 20 mg/kg) for 8 days increased SOD by 8.54% and 21.66%, respectively, compared to the GTN group. The quercetin control group showed a 3.5% increase over normal controls, while the vitamin E-treated (standard) group showed a 24.66% increase in SOD, higher than the quercetin groups.

GTN-treated rats showed a significant decrease in the concentration of SOD in the heart homogeneous samples, compared to normal controls. Treatment with quercetin and vitamin E daily for a period of eight days normalised the concentration of SOD in the serum sample compared to GTN-alone-treated rats. The SOD concentration was decreased by 41.67% in the GTN-treated group as compared with that of control animals. A rise in SOD concentration of about 24.16% and 52.02% was observed in the case of quercetin-treated groups at doses of 10 and 20 mg/kg, respectively, compared with the GTN control group. It was observed that there was a 4.95% increase in SOD concentration in the quercetin control group as compared with that of the control group, and a 63.42% increase. An increase in SOD concentration was found in the standard group compared with the GTN control group.

4.1.2 Concentration of MDA in Serum and Heart Homogenate Samples of Different Treated Animals

GTN-treated rats showed a 42.16% increase in serum MDA levels compared to controls. Pre-treatment with quercetin (10 and 20 mg/kg) for 8 days reduced MDA levels by 15.5% and 26.05%, respectively, compared to the GTN group. The quercetin control group showed a 9.89% decrease in MDA compared to normal controls, while the standard group (vitamin E) showed a 24.17% decrease compared to both GTN and control groups.

GTN-treated rats showed a significant increase in the concentration of MDA in the heart homogenate samples compared to normal controls. The MDA concentration was increased by 46.37% in the GTN-treated group as compared with that of control animals. It was shown that there is a decrement in MDA concentration of about 8.1% and 24.21% in the case of quercetin-treated groups at doses of 10 and 20 mg/kg, respectively, compared with that of the GTN control group. It was observed that there was an 18.95% decrease in MDA concentration in the quercetin control group, as compared with that of control and 27.68%, decrease in MDA concentration was found in the standard group than that of GTN control group.

4.1.3 Concentration of Total Protein Content in Serum and Heart Homogenate Samples of Different Treated Animals

GTN-treated rats showed a 23.15% decrease in serum Total Protein Concentration (TPC) levels compared to controls. Pre-treatment with quercetin and vitamin E for 8 days normalised TPC levels. Vitamin E led to higher TPC levels than quercetin. Quercetin at 10 and 20 mg/

kg increased TPC by 27.51% and 39.37%, respectively, compared to the GTN group. The quercetin control group showed an 11.23% decrease versus normal controls, while the standard group showed a 45.5% increase compared to both GTN and control groups.

The TPC concentration was decreased by 35.1% in the GTN-treated group as compared with that of control animals. A rise in TPC consultation of about 25.72% and 41.61% in the case of quercetin treated groups at doses of 10 and 20 mg/kg respectively to that of GTN control group, it was observed that there was a 5.56% increase in TPC concentration in the quercetin control group as compared with that of control and 48.54% increase in TPC concentration was found in the standard group to that of GTN control group.

4.1.4 Concentration of γ-GluT in Serum and Heart Homogenate Samples of Different Treated Animals

Γ-GLUT levels increased by 55.29% in GTN-treated rats compared to controls. Quercetin treatment at 10 and 20 mg/kg reduced Γ-GLUT by 9.74% and 22.29%, respectively, versus the GTN group. The quercetin control group showed a 5.57% decrease compared to normal controls, while the standard (vit E) group showed a 23.53% reduction versus the GTN group.

The Γ-GLUT concentration was increased by 41.37% in the GTN-treated group as compared with the top control animals. It was shown that there is a decrement in Γ-GLUT concentration of about 7.67% and 15.67% in the case of quercetin treated groups at doses of 10 and 20 mg/kg respectively to that of GTN control group, it was observed that there was a 6.56% decrease in Γ-GLUT concentration in the quercetin control group as compared with that of control and 23.09% decrease in Γ-GLUT concentration was found in the standard group than that of GTN control group.

4.1.5 Concentration of Nitrite in Serum and Heart Homogenate Samples of Different Treated Animals

The nitrite concentration was increased by 260.44% in the GTN-treated group as compared with that of control animals. It was shown that there is a decrease in nitrite concentration of about 13.57% and 50.06% in the case of quercetin-treated groups at doses of 10 and 20 mg/kg, respectively, compared with the GTN control group. It was observed that there was a 4.67% increase in the nitrite concentration in the quercetin control group as compared with that of control and 45.96%, decrease in nitrite concentration was found in the standard group than that of GTN control group.

The nitrite concentration was increased by 125.02% in the GTN-treated group as compared with that of control animals. It was shown that there is a decrement in nitrite concentration of about 13.07% and 19.37% in the case of quercetin treated groups at doses of 10 and 20 mg/kg respectively to that of GTN control group, it was observed that there was a 27.49% increase in nitrite concentration in the verity in control group as compared with that of control and a 15.27% decrease in nitrite concentration was found in the standard group than that of GTN control group.

4.1.6 Concentration of Reduced Glutathione in Serum and Heart Homogenate Samples of Different Treated Animals

The GSH concentration was decreased by 46.42% in the GTN-treated group as compared with that of the control groups. A rise in GSH concentration of about 44.53% and 63.32% was observed in the case of quercetin-treated groups at doses of 10 and 20 mg/kg, respectively, compared with the GTN control group. It was observed that there was a 1.73% increase in GSH concentration in the quercetin control group as compared with that of control and 72.24% increase in GSH concentration was found in the standard group as compared with that of the GTN control group.

The GSH concentration was decreased by 32.67% in the GTN treated group as compared with that of control animals rise in GSH concentration of about 12.92% and 27.32% in the case of quercetin treated groups at doses of 10 and 20 mg/kg respectively to that of GTN control group, it was observed that there was a 3.38% increase in GSH concentration in quercetin control group as compared with that of control and a 38.765% increase in GSH concentration was found in the standard group to that of GTN control group.

Figures 1–6 show the effect of quercetin on SOD, MDA, TPC, Γ-GLUT, nitrite, and reduced GSH levels in heart homogenates of experimental rats. Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison

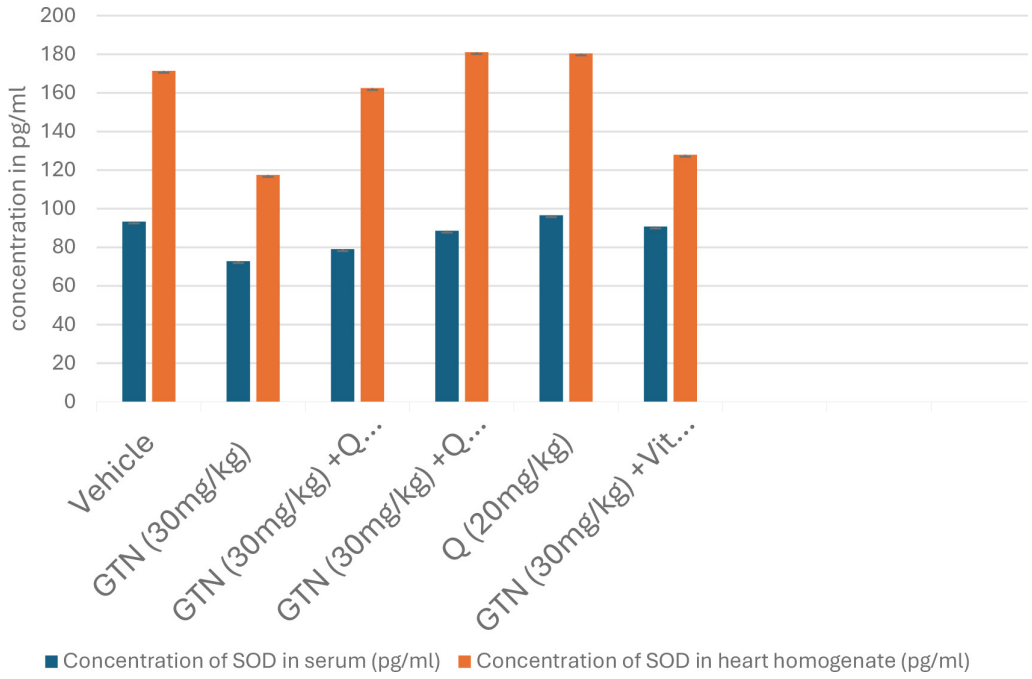


Figure 1. Effect of quercetin on SOD levels in serum and heart homogenates.

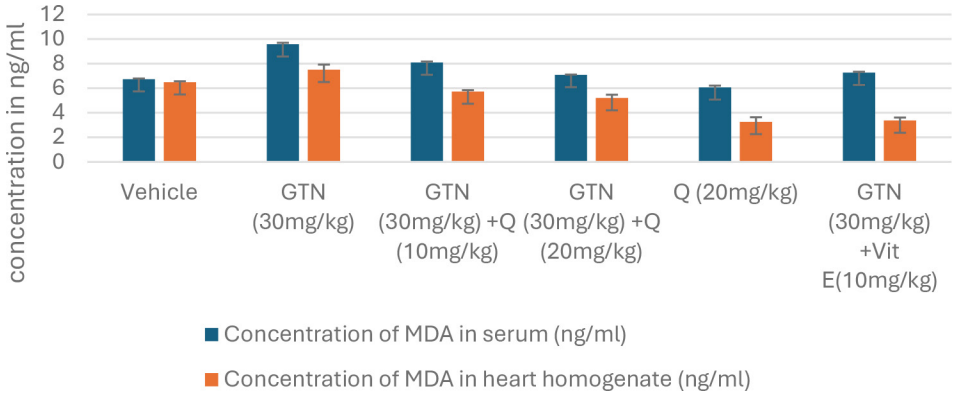


Figure 2. Effect of quercetin on MDA levels in serum and heart homogenates.

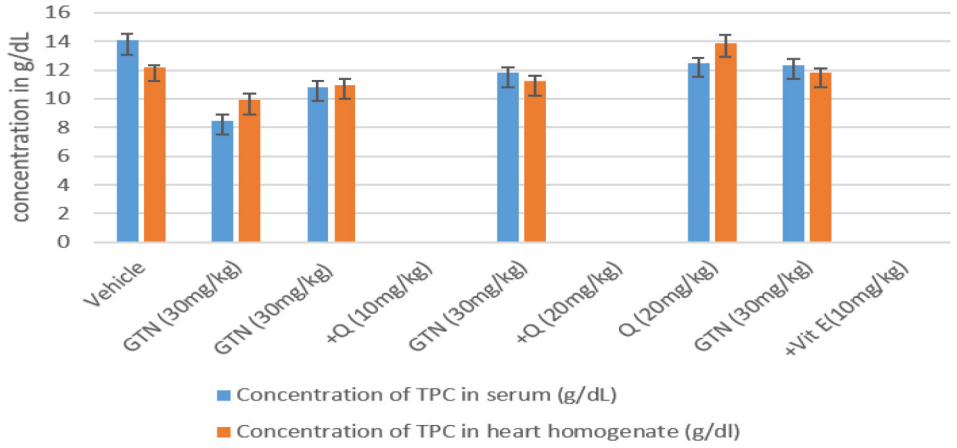


Figure 3. Effect of quercetin on total protein concentration in serum and heart homogenates.

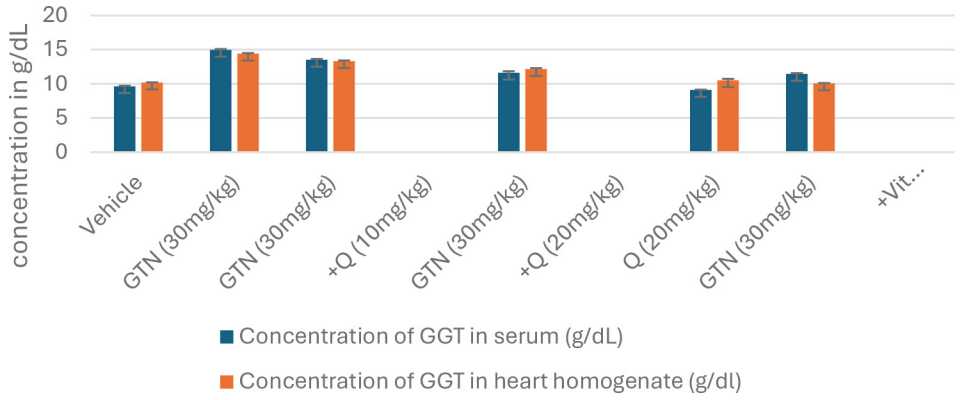


Figure 4. Effect of quercetin on Γ-GLUT activity in serum and heart homogenates

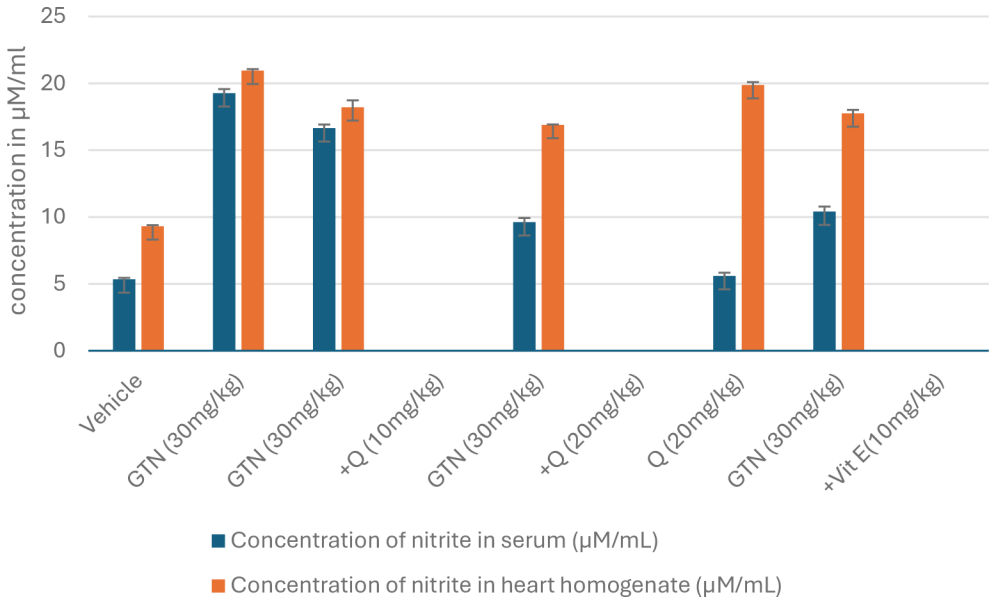


Figure 5. Effect of quercetin on nitrite concentration in serum and heart homogenates.

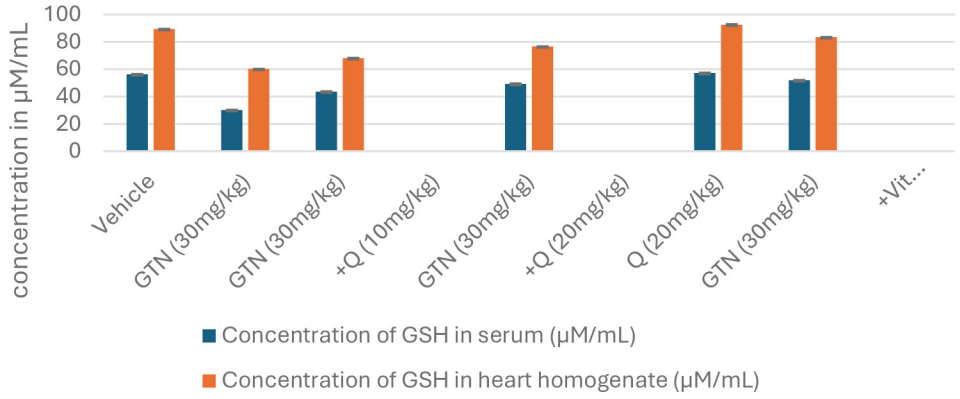


Figure 6. Effect of quercetin on GSH levels in serum and heart homogenates.

test. $P < 0.05$ indicate significant differences compared with the Vehicle control group, whereas #P < 0.05, ##P < 0.01, and ###P < 0.001 indicate significant differences compared with the GTN-treated group.

4.2 Statistical Analysis

All the values are expressed as mean ± SEM, n=6, ^=p<0.001 compared to group I (normal control), $^{\theta}$=p<0.001 compared to group II (GTN-treated group). Results were analysed by one-way ANOVA followed by Dunnett's multiple comparison tests. The statistical significance symbols and criteria used for intergroup comparisons are presented in Table 1.

4.3 Histological Studies

The media-lumen ratio of cardiac arteries was observed using computer-aided morphometry devices. Cardiac artery tissue samples were obtained from Wistar rats. Tissue specimens were fixed in 10% neutral-buffered formalin for 24–48 hours, followed by routine dehydration and paraffin embedding. Sections of 5µm thickness were cut using a rotary microtome. For histological evaluation, sections were stained with Hematoxylin and Eosin (H and E) and Verhoeff–Van Gieson (VVG) stains to visualise the lumen, Internal Elastic Lamina (IEL), and External Elastic Lamina (EEL). Slides were scanned using a light microscope equipped with a digital camera. Images were captured at 40x magnification, ensuring clear visualisation of vascular structures. Image acquisition was performed under identical lighting and exposure conditions for all samples. Manual/automated segmentation of the lumen, media, and external boundaries using the "Freehand selection" or "Threshold" tools[18]. Measured Lumen area and media area, followed by Media: Lumen Ratio = media/lumen.

Table 1. Statistical significance symbols and criteria used for comparison among experimental groups in the nitroglycerin (GTN)-induced nitrate tolerance study

Configured to Normal	Configured to GTN
^{a}=P<0.05	*= P<0.05
^{b}=P<0.01	$^{\#}$= P<0.01
$^{\theta}$=P<0.001	^=P<0.001

The media-to-lumen ratio of GTN-treated animals was increased significantly compared to controls. Treatment with quercetin completely abolished this effect. The diameter of cardiac myocytes was accordingly observed. It was discovered that in both GTN-treated groups, the mean diameter of cardiomyocytes

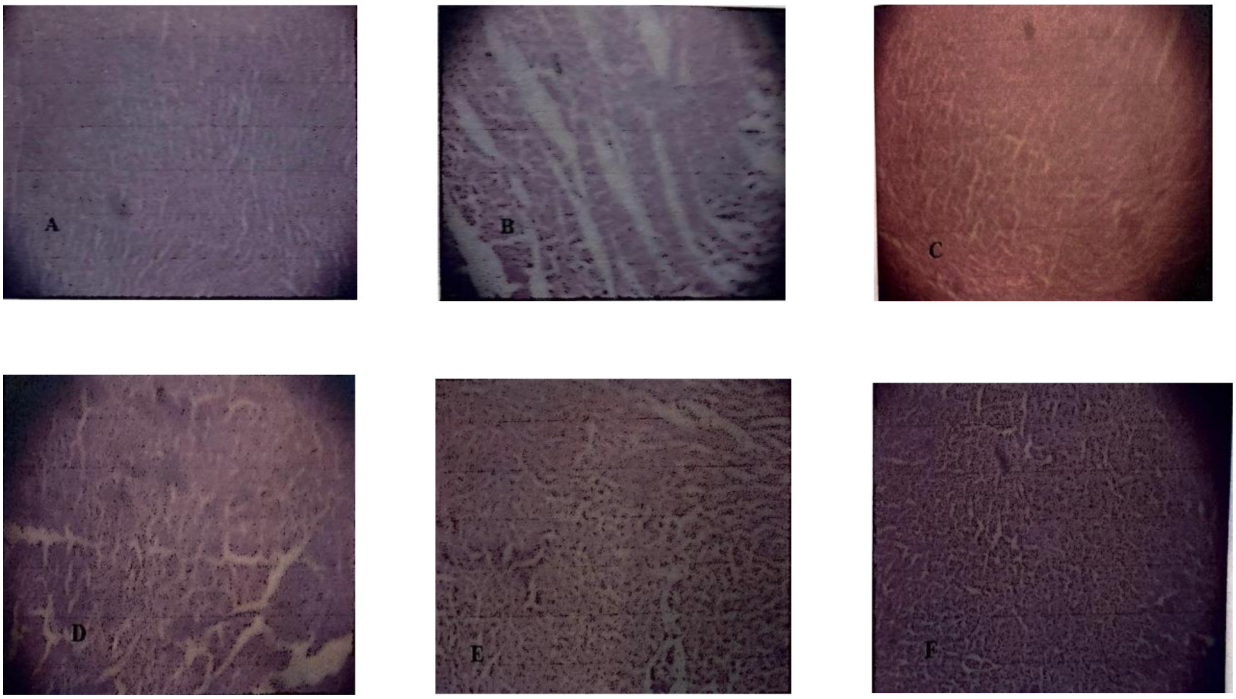


Figure 7. Representative photomicrographs of cardiac histology showing the effect of quercetin on GTN-induced nitrate tolerance in albino Wistar rats. **(A).** Vehicle-treated heart; **(B).** GTN (30 mg/kg)-treated heart; **(C).** GTN (30 mg/kg) + Quercetin (10 mg/kg)-treated heart; **(D).** GTN (30 mg/kg) + Quercetin (20 mg/kg)-treated heart; **(E).** Quercetin (20 mg/kg)-treated heart; **(F).** GTN (30 mg/kg) + Vitamin E (10 mg/kg)-treated heart.

increased significantly versus controls, but treatment with quercetin effectively diminished this increase.

Perivascular and interstitial fibrosis of the hearts were observed in Sirius-Red-staining[19]. Cardiac tissue samples were fixed in formalin to preserve the structure. The samples were then dehydrated through a series of alcohol solutions and were embedded in paraffin. Sections of the heart were cut at around 5-10 µm thickness using a microtome. The sections were stained with Sirius Red, which binds specifically to collagen fibres. The tissue was then treated with picric acid to differentiate collagen from other structures. Under a microscope, areas of fibrosis were shown as increased collagen deposition in the perivascular spaces (around blood vessels) and in the interstitial areas (between muscle fibres), which can be quantified.

Prolonged use of GTN can lead to increased oxidative stress, which can damage endothelial cells and smooth muscle cells in the vasculature. This oxidative stress is thought to stimulate fibroblasts to produce collagen and other extracellular matrix proteins, leading to fibrosis around blood vessels[20].

In rats, quercetin has been explored for its effects on vascular remodelling and fibrosis in the context of nitrate tolerance. It was found that quercetin treatment may mitigate the perivascular fibrosis induced by GTN, preserving vascular compliance and function. Quercetin has been shown to improve endothelial function and enhance NO bioavailability, which could counteract the endothelial dysfunction induced by nitrate tolerance[21].

Histopathological examination revealed that GTN-treated rats exhibited marked structural alterations, including an increased media-to-lumen ratio and cardiomyocyte hypertrophy. These pathological changes were markedly attenuated by quercetin treatment, particularly at 20 mg/kg, with the cardiac architecture approaching that of the vehicle-treated group (Figure 7).

5. Discussion

Oxidative stress, defined as an imbalance between ROS production and antioxidant defences, plays a pivotal role in nitrate tolerance. NO derived from organic nitrates undergoes rapid scavenging by free radicals, leading to diminished vasodilatory efficacy[20]. Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase and uncoupled endothelial NO synthase have been implicated as major sources of superoxide radicals, which interact with vascular NO to form peroxynitrite. This, in turn, exacerbates nitrate tolerance by inhibiting NO synthase and soluble guanylyl cyclase activity.

The findings suggest that chronic exposure to high doses of GTN provides a robust model for studying nitrate tolerance mechanisms. Furthermore, liposomal SOD administration improved both GTN- and acetylcholine-induced vasorelaxation, reinforcing the role of oxidative stress in limiting GTN's vasodilatory potential.

Notably, treatment with GTN led to a substantial decline in vascular SOD activity, attributed to reduced protein expression. This highlights an additional mechanism by which prolonged GTN therapy exacerbates oxidative stress. However, quercetin, a potent flavonoid antioxidant, effectively neutralised free radicals, as demonstrated by increased SOD levels in serum and cardiac homogenates from rats treated with 10 and 20 mg/kg quercetin alongside GTN.

The present study investigated the modulatory effects of quercetin, a potent natural flavonoid, on GTN-induced nitrate tolerance in albino Wistar rats. Chronic GTN administration is known to induce oxidative stress, leading to a reduction in NO bioavailability and vascular responsiveness, ultimately resulting in nitrate tolerance. The findings from this study support the hypothesis that quercetin co-administration can significantly ameliorate nitrate tolerance, primarily via its antioxidant and endothelial-protective effects.

Chronic nitrate use increases oxidative stress, which can lead to endothelial Nitric Oxide Synthase (eNOS) uncoupling. When uncoupled, eNOS produces superoxide instead of nitric oxide, worsening oxidative damage and impairing NO bioavailability.

Continuous GTN use leads to desensitisation of the nitrate–NO–cGMP pathway. This is compounded by an increase in ROS production, mitochondrial dysfunction, and depletion of endogenous antioxidants like GSH. These pathological alterations collectively impair vasodilation. In this model, rats receiving chronic GTN exhibited significantly elevated MDA levels, indicating lipid peroxidation, along with reduced GSH levels and decreased activities of antioxidant enzymes (e.g., superoxide dismutase, catalase).

Co-treatment with quercetin markedly improved these biochemical markers. GSH levels were restored toward normal values, and MDA levels were significantly reduced compared to the GTN-only group. This suggests that quercetin neutralised free radicals generated by GTN and protected myocardial tissue from oxidative damage. Quercetin is rich in hydroxyl groups that make it particularly effective in scavenging ROS and chelating metal ions that catalyse oxidative reactions.

Preservation of Cytochrome P450 Enzymes: Nitrate tolerance is associated with decreased expression of cardiac cytochrome P450 enzymes, which are involved in the biotransformation of nitrates. Vitamin E supplementation helps maintain the activity of these enzymes, thereby enhancing the conversion of nitrates to their active metabolites.

Additionally, quercetin may enhance eNOS expression and activity, promote NO bioavailability and improve vascular responsiveness. This could contribute to restoring the vasodilatory effects of GTN, thus reversing tolerance. Histopathological analyses (if included in your study) likely support this conclusion, showing preserved myocardial architecture in quercetin-treated animals compared to GTN-alone groups.

These outcomes are consistent with previous studies illustrating the cardioprotective effects of quercetin through multiple mechanisms, along with the reduction of oxidative stress, restoration of antioxidant enzyme activity, and enhancement of endothelial function[22–24].

These findings collectively indicate that quercetin acts through multiple mechanisms to attenuate the development of nitrate tolerance: antioxidant defence, preservation of redox balance, and support of NO signalling.

6. Conclusion

The present study demonstrates that quercetin effectively mitigates GTN-induced nitrate tolerance in albino rats. Chronic administration of GTN led to significant biochemical alterations, including elevated oxidative stress markers and impaired endogenous antioxidant defence mechanisms. Co-treatment with quercetin significantly restored antioxidant enzyme levels and reduced oxidative damage, suggesting its potent free radical scavenging and cardioprotective properties.

These findings support the therapeutic potential of quercetin as an adjunct to nitrate therapy, potentially improving the efficacy of long-term GTN treatment by preventing tolerance development. Further studies are warranted to explore the molecular mechanisms involved and to evaluate its clinical applicability.

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