Journal of Advanced Biomedical and Pharmaceutical Sciences

Journal Homepage: http://jabps.journals.ekb.eg

Dihydromyricetin Alleviates Gentamicin Induced Vascular Dysfunction through Inhibition of ROS/NF-κB Activation

Aliaa Anter[1], Eman M. Awad[1], Amr A. Kamel[1,2], Asmaa I. Matouk[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Minia University, Minia, Egypt
[2]Department of Physiology and Pharmacology, Schulich school of medicine and dentistry, University of Western Ontario, London Ontario, ON, Canada

Received: February 20, 2023; revised: March16, 2023; accepted: March 21, 2023

Abstract

Gentamicin causes an impairment of vascular function due to endothelial cell damage. Renal injury by gentamicin may contribute to the development of vascular dysfunction due to decreased NO bioavailability as well as increased oxidative stress and inflammation. In this study we investigate the role of DHM on vascular dysfunction induced by gentamicin. Male Wistar rats were divided into 3 groups (n= 6, each); control group; received the vehicle, gentamicin group; given gentamicin (100 mg/kg/day i.p) for 8 days and gentamicin + dihydromyricetin group; rats were treated with dihydromyricetin (200 mg/kg, p.o) concurrently with gentamicin. DHM reversed the GTN-induced histopathological changes of aortic rings. In addition, administration of DHM to GTN treated rats improved the vascular functions evident as enhanced vasorelaxation and vasoconstriction responses to acetylcholine and phenylephrine, respectively. Further, DHM increased the vascular levels of GSH and total antioxidant activity. Immunohistochemical analysis of aortic sections revealed that DHM treatment downregulated NF-κB, TNF-α and caspase-3 expression. Our data provide a new evidence for the protective effects of DHM against gentamicin-induced vascular dysfunction via provoking antioxidant, anti-inflammatory and antiapoptotic effects.

Keywords

DHM, endothelial. Nitric oxide, GSH, caspase-3, NF-κB, TNF-α and caspase-3

1.Introduction

Aminoglycosides, especially gentamicin (GTN), are widely used antibiotics against gram-negative bacterial infections because of their efficacy and low cost. Unfortunately, the clinical use of gentamicin is limited due to its nephrotoxic side effect [1]. Oxidative stress and inflammation have been considered the main causes of gentamicin induced renal damage [2, 3]. However, there is a growing interest in the role of vascular dysfunction in the pathogenesis of GTN-induced nephrotoxicity since decreased renal blood flow (RBF) results in decreased glomerular filtration rate and impairs renal function. Further, deprivation of renal tubules from oxygenated blood initiates their damage and death [4, 5]. Gentamicin increases the release of vasoconstrictors as endothelin-1 and thromboxane A2 while decreases the release of the vasodilator prostaglandins. Further, gentamicin induces a vascular oxidative stress that damages the endothelial cells leading to impaired vascular reactivity and consequently decreased renal perfusion and induced renal damage [6-8]. On the other side, research correlated renal failure to the development of endothelial dysfunction since many vascular related disorders as hypertension and cardiac diseases may develop with renal dysfunction [9, 10]. Likewise, renal injury induced by gentamicin could attenuate the vascular endothelium function. Evidence showed that gentamicin reduces renal levels of arginine, the rate limiting substance for nitric oxide synthase, leading to decreased nitric oxide (NO) [11]. The latter is derived from the endothelium and is essential for vasodilation, inhibition of platelet aggregation and inhibition of vascular smooth muscle proliferation. Decreased NO bioavailability disturbs the vascular homeostasis and initiates endothelial dysfunction [12]. Whether vascular dysfunction either arises from GTN-induced nephrotoxicity or magnifies the renal damage by GTN, it is essential to find a therapeutic agent that could counteract the GTN-induced vascular changes.

Dihydromyricetin (DHM) is a plant flavonoid derived from a traditional Chinese medicinal plant *Ampelopsis grossedentata* (*A. grossedentata*) [13]. DHM like other flavonoids possesses antioxidant, anti-thrombotic, anti-tumor and anti-inflammatory effects [14]. Interestingly, DHM could protect against diabetic as well as hydrogen peroxide induced endothelial injury. Further, DHM inhibited the development of atherosclerosis and increased endothelial NO production. DHM has cardioprotective effects on myocardial ischemia reperfusion (I/R) model [15], myocardial remodeling, arrhythmia and pulmonary artery hypertension [16]. DHM also attenuates insulin resistance and improves diabetic cardiomyopathy [16,17].

**** Correspondence:*** *Asmaa I. Matouk*
Tel.: *+201121744840*
Email Address: *asmaa.ahmed@mu.edu.eg*

The present study explored the pathophysiological pathways mediating the gentamicin-induced vascular dysfunction and investigated whether treatment with DHM concurrently with GTN could protect vascular endothelium from injury.

2. Materials and Method

2.1 Animals

Eighteen male Wistar rats weighing (200–220 g) were purchased from the animal care unit of El-Nahda University (Beni-Suef, Egypt). Animals were kept under standard conditions (12 h light/ 12 h dark cycle) and a temperature of 22 ± 2 ◦C for 7 days before the experiment. Animals were kept with free access to food and water throughout the experiment. This study was approved by the "Commission on the Ethics the of Scientific Research", Faculty of Pharmacy, Minia University, Code Number: ES33/2021

2.2. Chemicals

Gentamicin sulphate was purchased from (Schering–Plough, Cairo, Egypt). DHM was purchased from (BulkDHM, London, UK). Commercial kits including; reduced glutathione (GSH) and total antioxidant were obtained from (Biodiagnostic, Cairo, Egypt).

2.3. Experimental design

Animals were randomly divided into three groups as follow: Control group: rats were injected 0.5 ml normal saline, i.p. and given carboxymethyl cellulose, p.o., 0.5 % w/v (vehicle of DHM). GTN group: rats were injected gentamicin (100 mg/kg/day, i.p) [2, 18] and given 0.5 % w/v carboxymethyl cellulose p.o. GTN+DHM: animals were injected by gentamicin (100 mg/kg/day, i.p.) and received DHM (200 mg/kg/day, p.o.) [19]. All drugs are freshly prepared and given daily for eight days.

2.4. Tissue sampling

At the end of study, 24 hours after last dose administration, animals were anesthetized with urethane (1 g/kg, i.p.) then animals were sacrificed by decapitation. Aorta was rapidly dissected from each rat and cut into pieces, one was used for studying endothelial function, another was flash frozen in liquid nitrogen for further biochemical analysis and finally a piece was prepared for histopathological and immunohistochemistry examination. Aortic tissue used for bioassay was carefully and rapidly transferred to ice-cold Krebs-Henseleit buffer solution, previously aerated with Carbogen gas (95% O_2 and 5% CO_2). Blood was centrifuged at 3500 rpm for 10 min then serum samples were collected and stored for determination of creatinine and urea levels. Supernatants were collected after centrifugation of aortic homogenates were used for assessment of oxidative stress.

2.5. Examination of histopathological changes in aortic tissues

The aortic tissues were fixed in 10% neutral formalin and dehydrated in ascending grades of ethanol then cleared in xylol. Paraffin blocks were prepared and serial sections of 6 µm were prepared and stained with H&E [20]. The slides were examined and photographed using Olympus microscope in the Histology Department Minia University.

2.6. Assessment of vascular function of isolated aortic tissues

The thoracic aorta was dissected and cut into rings about 3mm length after removal of loose connective tissue. The vascular segments were then mounted between two stainless steel triangular hooks and transferred to organ baths (Panlab, Spain) of 10 ml capacity containing Krebs-Henseleit solution with the following composition(in mmol/l): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.0, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 11.1, pH 7.45) at 37°C. One end of the mounting assembly was connected to isometric force displacement transducers (Panlab MLT0202, Spain), connected to AD Instruments Powerlab4/35 for data acquisition. Tension was gradually adjusted to reach 2.0 g, and applied for 60 minutes, after which aortic rings were stimulated by 80 mmol/l KCl to test for viability. To test endothelial contractility, the aortic rings challenged with phenylephrine (PE: 1 µmol/l). Acetylcholine (Ach: 10-8 ~ 10 -4 M) was added in an increasing cumulative manner to record the endothelial-dependent relaxation. Percent relaxation was calculated as a change in tension after addition of relaxing agent acetylcholine divided by the maximal tension reached by the maximal tension reached by PE and multiplied by 100. The procedures were repeated using sodium nitroprusside (1 nM- 3 nM) in cumulative manner to test the endothelial independent relaxation.

2.7. Determination of oxidative stress and antioxidant capacity

Oxidative stress was determined in aortic homogenates by measuring nitric oxide (NO) levels by a colorimetric Griess assay method which is based on determination of total nitrate levels after reduction of nitrite to nitrate [21]. Glutathione and total antioxidants were evaluated in aortic homogenates using commercial kits based on manufacturers' instructions.

2.8. Immunohistochemical analysis of aortic NF-κB, TNF-alpha, caspase-3 levels

In order to determine the aortic levels of NF-κB, TNF-alpha, caspase-3, we used streptavidin–biotin immunoperoxidase method. The aortic sections were first deparaffinized with xylene and hydrated by decreasing concentrations of ethanol. After incubation for 20 min in a solution of 3% H_2O_2 in water to inhibit endogenous peroxidase activity, they were washed (3×10 min) in PBS (0.01 mol/l, pH 7.4). Nonspecific binding sites for immunoglobulins were blocked by 15-min-incubation with 0.25% casein in PBS. The sections were then washed in PBS and incubated with the polyclonal primary antibodies including; Anti-NF-κB (1:250); (Catalogue number# ab32360, Rabbit monoclonal, Abcam, USA), Anti- TNF-α antibody (1:10); (Catalogue number# ab215188, Rabbit monoclonal, Abcam, USA) and Anti-caspase-3 (1:10); (Catalogue Number# AB3623, rabbit polyclonal antibodies, Merck KGaA, Darmstadt, Germany). The slides were subsequently washed (3×10 min) in PBS. The immunohistochemical visualization was carried out using the Ready-to-Use Immunostaining Kit (QD000-5L; BioGenex, San Ramon, California, USA) at 20°C. The sections were incubated for 30 min with biotinylated anti-IgG and finally washed in PBS. The reaction site was revealed with 100 µl

diaminobenzidine tetrahydrochloride chromogen solutions in 2.5 ml PBS and 50 µl H2O2 substrate solution, resulting in a brown precipitate. The sections were counterstained with hematoxylin [22]. Morphometric estimation using Leica QWin 500 image analysis software (Leica Microsystems, Wetzlar, Germany) was used [23] using power X 400. The mentioned parameters were detected in 10 non overlapping sections from each slide from each rat from each group. Semiquantitative scoring of aortic lesions was calculated according to Gibson-Corley et al. [24]. Briefly, lesions in 10 fields were chosen randomly from each slide for each rat and averaged. The lesions were scored in a blinded way (score scale: 0 = normal; 1 ≤ 25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%).

3. Results

3.1. Histopathological Examination

Control group showed normal tunica intima, tunica media, and tunica adventitia (Fig.1A). Gentamicin administration displayed thin tunica intima, tunica media, and tunica adventitia. Also, the separated fibers of tunica media were observed (Fig.1B).

GTN + DHM group showed apparent normal tunica intima, tunicamedia, and tunica adventitia. Less separated fibers of tunica media were seen (Fig.1C).

3.2. DHM ameliorated the GTN-induced changes in vascular functions

To determine the changes in vascular response toward contraction, isolated aortic rings of all groups were exposed to phenylephrine in different concentrations. Comparing to control rats, which showed a concentration dependent contraction in response to phenylephrine, the GTN treated rats significantly ($p< 0.05$) attenuated the aortic vasoconstriction with increasing the phenylephrine concentration (Fig. 2A).
However, DHM treatment significantly ($p< 0.05$) abrogated the GTN induced changes in aortic vasoconstriction (Fig. 2A). On the other hand, GTN caused a significant ($p< 0.05$) decrease in aortic vasorelaxation in response to the endothelium dependent vasorelaxant, acetylcholine (Fig. 2B). In contrast, DHM significantly ($p< 0.05$) ameliorated the GTN –induced decreased vasorelaxant response to acetyl choline (Fig. 2B). On the other side, sodium nitroprusside (an endothelium-independent vasodilator) caused a concentration-dependent relaxation with the maximal relaxation was not changed among the three groups; control, GTN and GTN+ DHM as shown in (Fig. 2C).

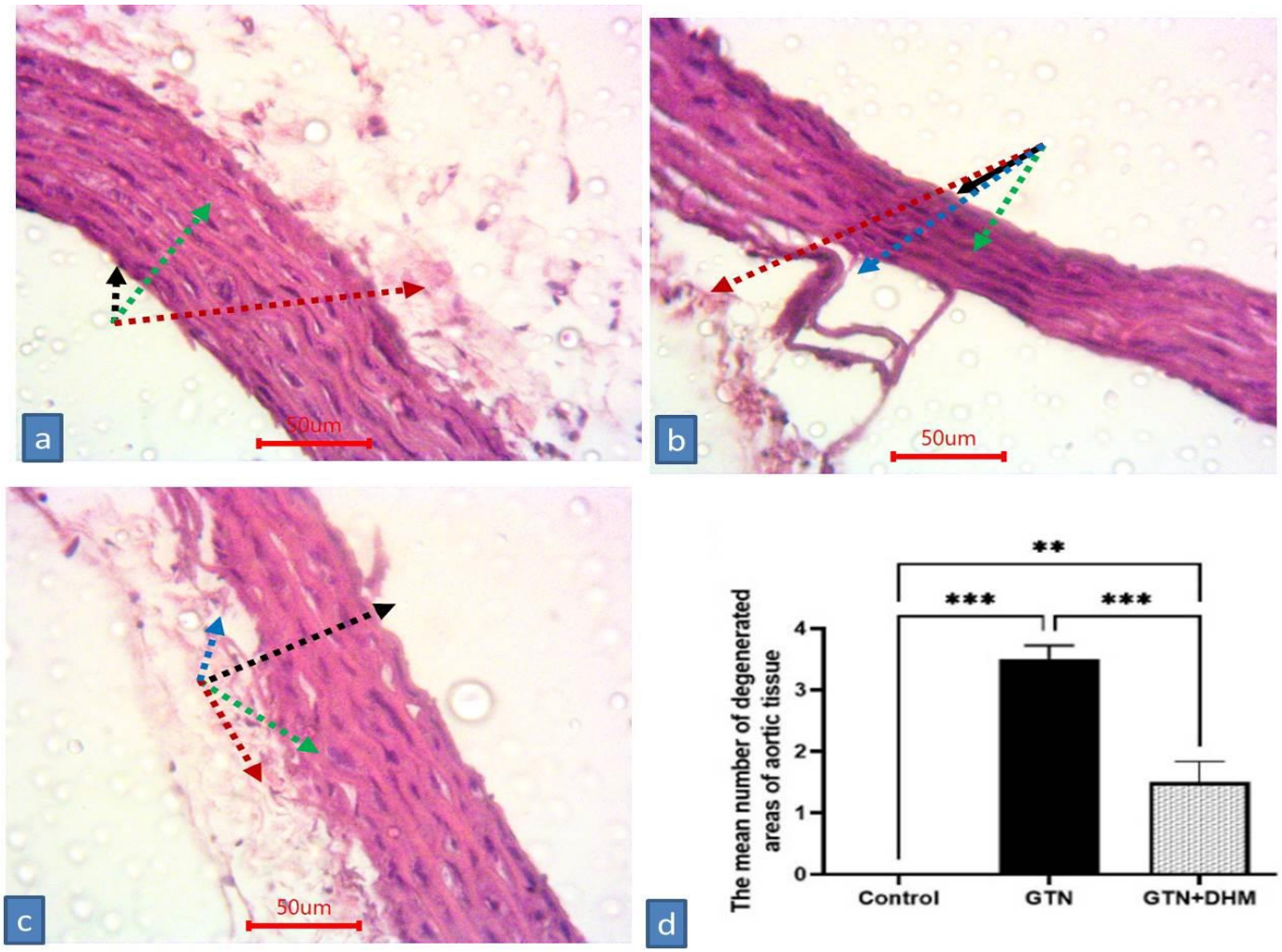

Figure 1. Photomicrographs of rat aorta, control group (a): showing normal tunica intima (black arrow), tunica media (green arrow), and tunica adventitia (red arrow). GTN (b): showing thin tunica intima (black arrow), tunica media (green arrow), and tunica adventitia (red arrow). Notice the separated fibers of tunica media (blue arrow). GTN+DHM (c): showing apparent normal tunica intima (black arrow), tunica media (green arrow), and tunica adventitia (red arrow). Notice the less separated fibers of tunica media (blue arrow). (d): Bar chart showing H&E semiquantitative scoring of degenerated areas of aortic tissue. Data are expressed as mean ± S.E. ** and *** are significant differences between the selected study groups at p values < 0.01 and 0.001, respectively

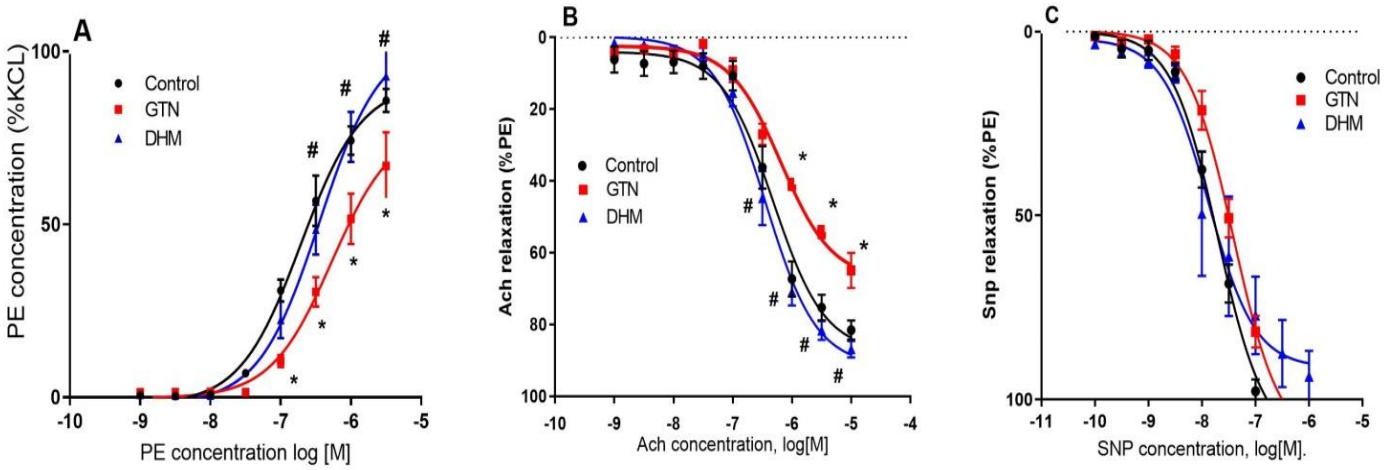

Figure. 2. Effect of DHM on GTN-induced changes in vascular reactivity. (A) shows the cumulative concentration-response curves of phenylephrine (PE)-induced contraction (B) shows the cumulative concentration-response curves of acetylcholine (ACh)-induced relaxation and (C) shows the cumulative concentration-response curves of sodium nitroprusside-induced relaxation. * Significant compared to control group at $p < 0.05$, # Significant compared to GM group at $p < 0.05$. Data represent the mean ± SEM of at least 6 independent experiments. Two-way ANOVA followed by Bonferroni test was used for statistical analysis

3.3. Effect of Dihydromyricetin on GTN-induced increase of vascular oxidative stress

Total nitrite level was used as an indicator of nitric oxide bioavailability. Nitrite level was significantly ($p < 0.05$) increased in rats treated with gentamicin compared to control group (Fig. 3 A). In contrast, administration of DHM concurrently with GTN resulted in a significant ($p < 0.05$) decrease in aortic NO when compared to GTN treated group (Fig 3A). Furthermore, the levels of glutathione and total antioxidants in the aortic homogenates were significantly ($p < 0.05$) declined upon GTN administration (Fig. 3 B and 3C). Conversely, DHM treatment restored the levels of glutathione and total antioxidants with non-significant difference with control aortic rats (Fig 3B and 3C).

3.4. DHM downregulated the GTN-induced elevation of inflammatory and apoptotic mediators in aortic tissues

Immunohistochemical analysis of aortic sections demonstrated a significant ($P < 0.05$) upregulation of caspase-3, a master regulator of apoptosis, in GTN treated rats (Fig. 4 A-D). Further, upon GTN administration, we reported a significant ($P < 0.05$) increase in aortic levels of NF-κB (Fig. 4 E-H) and the proinflammatory cytokine, TNF-α (Fig. 4 I-L). Concurrent use of DHM with GTN significantly attenuated the GTN induced rise in caspase-3, NF-κB and TNF-α levels in aortic tissues (Fig. 4)

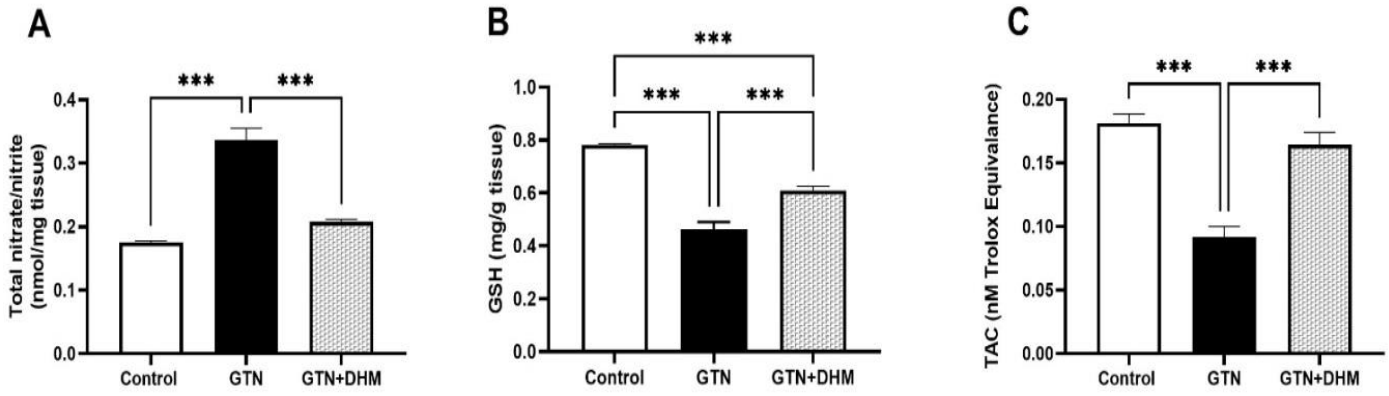

Figure 3. Effect of DHM on oxidative stress. A: bar chart showing total nitrate/nitrite concentration in aortic homogenates of Control, GTN and GTN+DHM groups B: bar chart showing Glutathione concentration in aortic homogenates of Control, GTN and GTN+DHM groups C: bar chart showing total antioxidants concentration in aortic homogenates of Control, GTN and GTN+DHM groups. Data are expressed as mean ± S.E. *, ** and *** are significant differences between the selected study groups at p values < 0.05, 0.01 and 0.001, respectively. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test.

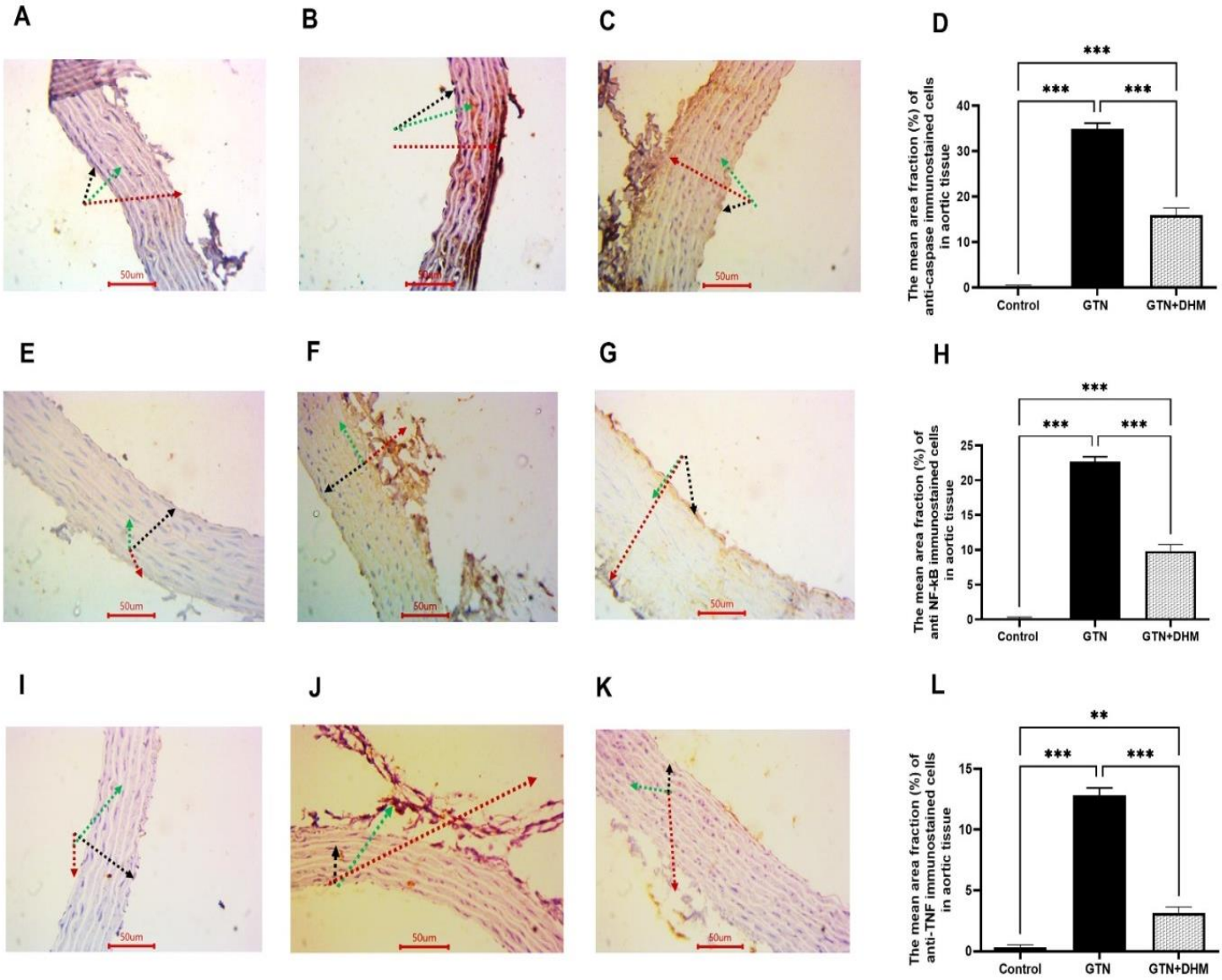

Figure 4. Effect of DHM treatments on vascular immunoreactivity to caspase-3), NF-kB and TNF-α. **A, B, C:** Representative photomicrographs show immunohistochemical staining of Caspase-3 in aortic tissue **D:** Quantitative analysis of caspase-3 expression in rat kidneys. **E, F, G:** Representative photomicrographs show immunohistochemical staining of NF-κB in aorta, **H:** Quantitative analysis of NF-κB expression in aorta. **I, J, K:** Representative photomicrographs show immunohistochemical staining of TNF-α in aorta, L: Quantitative analysis of TNF-α expression in aorta. GTN; gentamicin, DHM; dihydromyricetin. Data are expressed as mean ± S.E. *, ** and *** are significant differences between the selected study groups at p values < 0.05, 0.01 and 0.001, respectively. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test.

4. Discussion

The endothelium plays the main role in regulation of vascular tone. The endothelium is responsible of synthesizing and releasing different endothelium relaxing factors, such as nitric oxide, vasodilator prostaglandins, and endothelium-dependent hyperpolarization factors, as well as endothelium contracting factors. Endothelial dysfunction is mainly caused by reduced production or diminishes the action of relaxing mediators .[25] The present study provided evidence that GTN induces vascular toxicity through increasing oxidative stress, inflammatory and apoptotic mediators. However, treatment with DHM, the antioxidant flavonoid, counteracted all these pathways and protected against the GTN-induced vascular damage. Here, administration of GTN (100 mg/kg/day) for 8 days displayed a marked damage of aortic tissues as was shown by the histopathological examination. All layers of the aorta including; tunica intima, tunica media and tunica adventitia appeared thin than the normal. In parallel with this finding, GTN impaired the vascular reactivity; the isolated aortic rings of GTN treated rats showed a reduced ability to relax in response to the endothelium dependent vasodilator, acetylcholine and to contract in response to phenylephrine. However, their relaxation was not affected upon the addition of sodium nitroprusside, an external non endothelium dependent vasorelaxant. Our data are in accord with the previous studies and indicates that GTN causes an impairment of endothelium dependent vasorelaxation [11, 26]. A possible explanation of GTN-induced vascular dysfunction is impaired NO bioavailability in consequence to increased ROS production. GTN diminished the total antioxidant activity and attenuated the antioxidant, GSH, enzyme levels in aortic tissues

while, NO levels were markedly elevated. It is likely that GTN-induced ROS overproduction stimulates the expression of inducible nitric oxide synthase (iNOS) leading to high NO levels [27]. Our finding, in line with other studies, suggests the role of oxidative stress and NO in gentamicin mediated cytotoxicity [2, 28, 29]. Literature confirmed that, under physiological conditions, NO is predominantly released by endothelial nitric oxide synthase enzyme (eNOS) causing vasorelaxation and maintaining of the endothelial function. However, in response to oxidative stress and inflammation, iNOS-produces excess NO which interacts with superoxide anion radicals forming a potent oxidizing agent, peroxynitrite [30]. The latter causes damage to vascular proteins and DNA as well as eNOS uncoupling. Also, iNOS decreases the effect of eNOS by competing for BH4 and decreasing its availability [31,32].

On the other hand, DHM improved the histopathological changes of aortic tissues and mitigated the changes in vascular reactivity caused by GTN. DHM restored the ability of aortic rings to contract in response to phenylephrine. Further, DHM enhanced aortic vasorelaxation upon increasing acetyl choline concentration. However, it did not cause any change for the vascular response to nitroprusside. This finding indicates an enhancement of endothelial functions by DHM treatment. Several data has proved the protective effects of DHM against vascular function impairment; DHM ameliorated the diabetic induced vascular dysfunction as well as inhibited the development of atherosclerosis via attenuation of endothelial cell activation and damage. These beneficial vascular effects of DHM were found to be mediated via attenuation of ROS release [33-36]. Also, DHM was found to attenuate vascular iNOS/NO expression while enhances eNOS production leading to improved vascular activity [37, 38]. In the present study, the DHM mediated improved vascular reactivity is linked to elevated GSH and total antioxidant levels as well as rendering the elevated NO levels .

Besides to enhanced antioxidant effects, DHM decreased vascular levels of NF-κB, the transcription factor that positively regulate inflammatory cytokine release, and consequently reduced levels of the inflammatory mediator, TNF-α. Research proved that TNF-α induces vascular dysfunction via multiple pathways including; increasing ROS production, decreasing NO availability, vascular inflammation and vascular remodeling [39]. Thus, in agreement with others, we suggested that DHM-provoked anti-inflammatory effects play a central role in preserving the vascular functions [35, 38, 40]. Interestingly, DHM is considered a major natural NF-κB inhibitor. It downregulates NF-κB expression through inhibition of IKK phosphorylation, the rate limiting step in NF-κB phosphorylation and activation. Moreover, DHM blocks the degradation of (IkBa) which is an inhibitor of NF- κB activation [41, 42]. It is important to mention that, the upregulation of NF-κB and TNF-α expression in aortic tissues strongly matches the previously reported increase in their renal levels upon GTN administration. This indicates the role of inflammatory mediators in mediating GTN toxicity [2,43].

GTN-induced increase in oxidative stress, NF-κB and TNF- α triggered activation of caspase-3 in aortic tissues. Caspase-3 is a predominant mediator of apoptotic cell death through activation of DNA fragmentation and cellular protein degradation leading to damaged endothelial cells [44]. Alternatively, treatment with DHM inhibited vascular apoptosis and counteracted the GTN-mediated increase in caspase-3 levels. Similar to our findings, DHM protected the endothelial cells against H2O2-induced injury via suppression of the release of apoptotic mediators including caspase-3[38]. Researchers found that DHM exerted protective effects against cellular apoptotic death in endothelial cells as well as other organs by attenuation of ROS/ NF-κB signaling crosstalk [36, 45-47].

5. Conclusion

Our study suggests that DHM reversed the deleterious effects of GTN on vascular functions via inhibition of oxidative stress, inflammatory and apoptotic pathways. We suggested that DHM is potentially considered a protective natural product against GTN-induced impairment of vascular function.

Conflict of interest

There is no conflict of interest declared by the authors.

6. References

1. Jia, P., et al., Intermittent exposure to xenon protects against gentamicin-induced nephrotoxicity. PLoS One, 2013. 8(5): p. e64329.
2. Botros, S.R., et al., Protective effect of empagliflozin on gentamicin-induced acute renal injury via regulation of SIRT1/NF-κB signaling pathway. Environmental Toxicology and Pharmacology, 2022. 94: p. 103907.
3. Randjelovic, P., et al., Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. EXCLI J, 2017. 16: p. 388-399.
4. Hishida, A., et al., Roles of hemodynamic and tubular factors in gentamicin-mediated nephropathy. Ren Fail, 1994. 16(1): p. 109-16.
5. Martinez-Salgado, C., F.J. Lopez-Hernandez, and J.M. Lopez-Novoa, Glomerular nephrotoxicity of aminoglycosides. Toxicol Appl Pharmacol, 2007. 223(1): p. 86-98.
6. Valdivielso, J.M., et al., Increased renal glomerular endothelin-1 release in gentamicin-induced nephrotoxicity. Int J Exp Pathol, 1999. 80(5): p. 265-70.
7. Papanikolaou, N., et al., Does gentamicin induce acute renal failure by increasing renal TXA2 synthesis in rats? Prostaglandins Leukot Essent Fatty Acids, 1992. 45(2): p. 131-6.
8. Assael, B.M., et al., Prostaglandins and aminoglycoside nephrotoxicity. Toxicol Appl Pharmacol, 1985. 78(3): p. 386-94.
9. Mori-Kawabe, M., et al., Reduction of NO-mediated Relaxing Effects in the Thoracic Aorta in an Experimental Chronic Kidney Disease Mouse Model. J Atheroscler Thromb, 2015. 22(8): p. 845-53.
10. Nguy, L., et al., Vascular function in rats with adenine-induced chronic renal failure. Am J Physiol Regul Integr Comp Physiol, 2012. 302(12): p. R1426-35.
11. Aycan-Ustyol, E., et al., Vascular function and arginine and dimethylarginines in gentamicin-induced renal failure: a possible effect of heme oxygenase 1 inducer hemin. Can J Physiol Pharmacol, 2017. 95(12): p. 1406-1413.
12. Cyr, A.R., et al., Nitric Oxide and Endothelial Dysfunction. Crit Care Clin, 2020. 36(2): p. 307-321.
13. Liu, D., et al., Dihydromyricetin: A review on identification and quantification methods, biological activities, chemical stability, metabolism and approaches to enhance its bioavailability. Trends Food Sci Technol, 2019. 91: p. 586-597.
14. Zhang, J., et al., Recent Update on the Pharmacological Effects and Mechanisms of Dihydromyricetin. Front Pharmacol, 2018. 9: p. 1204.
15. Liu, S., et al., The cardioprotective effect of dihydromyricetin prevents ischemia-reperfusion-induced apoptosis in vivo and in vitro via the PI3K/Akt and HIF-1alpha signaling pathways. Apoptosis, 2016. 21(12): p. 1366-1385.
16. Li, Q., et al., Dihydromyricetin prevents monocrotaline-induced pulmonary arterial hypertension in rats. Biomed Pharmacother, 2017. 96: p. 825-833.
17. Wu, B., et al., Dihydromyricetin Protects against Diabetic Cardiomyopathy in Streptozotocin-Induced Diabetic Mice. Biomed Res Int, 2017. 2017: p. 3764370.
18. Mahmoud, A.M., et al., Agomelatine prevents gentamicin nephrotoxicity by attenuating oxidative stress and TLR-4 signaling, and upregulating PPARgamma and SIRT1. Life Sci, 2021. 278: p. 119600.
19. Sun, P., et al., Protective role of Dihydromyricetin in Alzheimer's disease rat model associated with activating AMPK/SIRT1 signaling pathway. Biosci Rep, 2019. 39.(1)

20. Bancroft, J.D. and C. Layton, The hematoxylins and eosin. Bancroft's Theory and Practice of Histological Techniques. Elsevier, 2013: p. 173-186.
21. Moorcroft, M.J., J. Davis, and R.G. Compton, Detection and determination of nitrate and nitrite: a review. Talanta, 2001. 54(5): p. 785-803.
22. Côté, S., Current protocol for light microscopy immunocytochemistry. Immunohistochemistry, II, 1993: p. 148-167.
23. Beshay, O.N., et al., Resveratrol reduces gentamicin-induced EMT in the kidney via inhibition of reactive oxygen species and involving TGF-β/Smad pathway. Life Sciences, 2020: p. 118178.
24. Gibson-Corley, K.N., A.K. Olivier, and D.K. Meyerholz, Principles for valid histopathologic scoring in research. Veterinary pathology, 2013. 50(6): p. 1007-1015.
25. Godo, S. and H. Shimokawa, Endothelial Functions. Arterioscler Thromb Vasc Biol, 2017. 37(9): p. e108-e114.
26. Secilmis, M.A., et al., Protective effect of L-arginine intake on the impaired renal vascular responses in the gentamicin-treated rats. Nephron Physiol, 2005. 100(2): p. p13-20.
27. Forstermann, U., N. Xia, and H. Li, Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circ Res, 2017. 120(4): p. 713-735.
28. Morsy, M.A., et al., Sildenafil Ameliorates Gentamicin-Induced Nephrotoxicity in Rats: Role of iNOS and eNOS. J Toxicol, 2014. 2014: p. 489382.
29. Ghaznavi, R. and M. Kadkhodaee, Comparative effects of selective and non-selective nitric oxide synthase inhibition in gentamicin-induced rat nephrotoxicity. Arch Toxicol, 2007. 81(6): p. 453-7.
30. Forstermann, U., Nitric oxide and oxidative stress in vascular disease. Pflugers Arch, 2010. 459(6): p. 923-39.
31. Korkmaz, S., et al., Nitric oxide- and heme-independent activation of soluble guanylate cyclase attenuates peroxynitrite-induced endothelial dysfunction in rat aorta. J Cardiovasc Pharmacol Ther, 2013. 18(1): p. 70-7.
32. Förstermann, U., N. Xia, and H. Li, Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. Circulation Research, 2017. 120(4): p. 713-735.
33. Hua, Y.Y., et al., Dihydromyricetin Improves Endothelial Dysfunction in Diabetic Mice via Oxidative Stress Inhibition in a SIRT3-Dependent Manner. Int J Mol Sci, 2020. 21.(18)
34. Yang, D., et al., Dihydromyricetin Attenuates TNF-alpha-Induced Endothelial Dysfunction through miR-21-Mediated DDAH1/ADMA/NO Signal Pathway. Biomed Res Int, 2018. 2018: p. 1047810.
35. Awad, E.M., et al., Dihydromyricetin protects against high glucose-induced endothelial dysfunction: Role of HIF-1alpha/ROR2/NF-kappaB. Biomed Pharmacother, 2022. 153: p. 113308.
36. Luo, Y., et al., Dihydromyricetin protects human umbilical vein endothelial cells from injury through ERK and Akt mediated Nrf2/HO-1 signaling pathway. Apoptosis, 2017. 22(8): p. 1013-1024.
37. Yang, D., et al., Dihydromyricetin increases endothelial nitric oxide production and inhibits atherosclerosis through microRNA-21 in apolipoprotein E-deficient mice. J Cell Mol Med, 2020. 24(10): p. 5911-5925.
38. Hou, X., et al., Dihydromyricetin protects endothelial cells from hydrogen peroxide-induced oxidative stress damage by regulating mitochondrial pathways. Life Sci, 2015. 130: p. 38-46.
39. Zhang, H., et al., Role of TNF-alpha in vascular dysfunction. Clin Sci (Lond), 2009. 116(3): p. 219-30.
40. Wang, X., et al., Dihydromyricetin alleviates endothelial inflammatory response through the IRE1α/NF-κB signaling pathway in sepsis. Archives of Medical Science, 2021.
41. Chen, Y., et al., Dihydromyricetin Attenuates Diabetic Cardiomyopathy by Inhibiting Oxidative Stress, Inflammation and Necroptosis via Sirtuin 3 Activation. Antioxidants (Basel), 2023. 12.(1)
42. Tang, N., et al., Dihydromyricetin suppresses TNF-alpha-induced NF-kappaB activation and target gene expression. Mol Cell Biochem, 2016. 422(1-2): p. 11-20.
43. Volpini, R.A., et al., Inhibition of nuclear factor-kappaB activation attenuates tubulointerstitial nephritis induced by gentamicin. Nephron Physiol, 2004. 98(4): p. p97-106.
44. Asadi, M., et al., Caspase-3: Structure, function, and biotechnological aspects. Biotechnol Appl Biochem, 2022. 69(4): p. 1633-1645.
45. Matouk, A.I., et al., Dihydromyricetin alleviates methotrexate-induced hepatotoxicity via suppressing the TLR4/NF-kappaB pathway and NLRP3 inflammasome/caspase 1 axis. Biomed Pharmacother, 2022. 155: p. 113752.
46. Wasan, H., et al., Dihydromyricetin alleviates cerebral ischemia-reperfusion injury by attenuating apoptosis and astrogliosis in peri-infarct cortex. Neurol Res, 2022. 44(5): p. 403-414.
47. Hu, Q., et al., Dihydromyricetin inhibits NLRP3 inflammasome-dependent pyroptosis by activating the Nrf2 signaling pathway in vascular endothelial cells. Biofactors, 2018. 44(2): p. 123-136.