EFFECT OF GALLIC ACID AGAINST HIGH GLUCOSE INDUCED VASCULAR DYSFUNCTION IN WISTAR RAT

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Summary

To evaluate the effect of gallic acid on vascular reactivity subsequent to high glucose induced stress in thoracic aorta of Wistar rat. The thoracic aortic rings with endothelium from wistar rats were mounted in an organ bath. Isometric relaxation of aortic rings was measured.

(1) After incubation with 44 mmol/L of high glucose for 4 h, the vascular relaxation response to acetylcholine (Ach) decreased in precontracted aortic ring with phenylephrine in an endothelium-dependent manner; (2) Coincubation with gallic acid (10^{-8}–10^{-2} mol/L) and high glucose, the high glucose-induced vasodilation dysfunction was significantly inhibited.

Gallic acid could protect the high glucose-induced acute endothelium dependent vascular dysfunction in rat aortic rings.

Keywords- Cyclosporine A, Gallic acid, Vascular dysfunction

Introduction

The endothelium plays a critical role in the regulation of vascular function. Previous evidences had suggested that high glucose can induce vascular endothelial damage through oxidative stress pathway by the mitochondrial electron-transport chain.[1]

Gallic acid (3, 4, 5-trihydroxybenzoic acid) is polyphenolic compound which is abundant in red wines, green tea, berry-berry, pomegranate, grapes, gallnuts, oak trees and chestnut.[2, 3] Gallic acids possess cytotoxicity against cancer cell[3], anti-inflammatory[3], anti-mutagenic[4], cardioprotective[5], neuroprotective[6], antioxidant[6], antiobesity and hepatoprotective[7]. Previous studies also suggested the protective effect of puerarin on high glucose induced vascular dysfunction[8].

However no study is being done for gallic acid .Thus, objective of current study is to observe the effect of gallic acid on high glucose induced acute vascular dysfunction.

Material and Method

Animal: Adult Male Wistar rats (220–270 g) were supplied by Experimental Animal Center of Jawahar Lal Nehru Medical College, Belgaum. The rats were allowed to free access to water and food ab libitum.

Drug: Phenylephrine (PE), Acetylcholine (Ach), Sodium Nitroprusside (SNP), Gallic Acid

Preparation of Gallic acid: Gallic acid was obtained from Sigma and is dissolved in normal saline which is adjusted to pH 7.4 by 1M NaOH solution.

Preparation of aortic ring: Male Wistar rats were sacrificed and thoracic aortas were quickly isolated and placed in cold Krebs–Henseleit (KH) solution and adjust to pH 7.4. The aorta rings was cut into (2–3 mm in length), then mounted in organ baths filled with warmed (37 °C) and oxygenated (95% O_2, 5% CO_2) KH solution. Isometric tension of 2 g was applied by a Biopac Data Acquisition System.

The rings were equilibrated for 60 min by continuous changing the KH solution at every 10 min interval. After the equilibration period, the rings were exposed to 60 mmol/L KCl for three times to evoke the maximal contraction. After this, pre contraction with cumulative dose of PE (10^{-8} to 10^{-2} mol/L), cumulative dose of ACh (10^{-8} to 10^{-2} mol/L) was added to the bath to check the integrity of the vascular endothelium. The successful removal of the endothelium was confirmed by the inability of tissues to relax in response to ACh. Further, the endothelium damage is confirmed when it is relaxed by adding SNP cumulatively.

Measurement of relaxant response in rat aortic rings: Aortic rings were precontracted with submaximal dose of PE (10^{-5} mol/L). Relaxant responses were expressed as the percentage decreases of the magnitude of the contraction induced by PE before the application of ACh. Maximum effect (E_{max}) and the inhibitory concentration 50% (IC50) were determined from concentration–response curve and pD2 was calculated as − log (IC50) respectively.

Experimental groups: Aortic rings ($n = 4$) were subjected with the following different treatments: (1) Control groups: With normal concentration of glucose (11 mmol/L) for 4 h; (2) High glucose (HG) groups: With high concentration of glucose (44 mmol/L) for 4 h; (3) Gallic acid (GA) groups: With Gallic acid (10^{-7} mol/L) for 4 h; (4) GA + HG groups: Both with high glucose and Gallic acid (10^{-7}, mol/L) for 4 h.

Statistical analysis: Data were expressed as mean ± SE and analyzed by one-way ANOVA with Bonferroni post hoc test as required. The probability value of 0.05 was accepted as significant for differences between groups of data.

Results

1.1 Effect of high glucose on Ach induced relaxation in rat aortic rings with intact endothelium

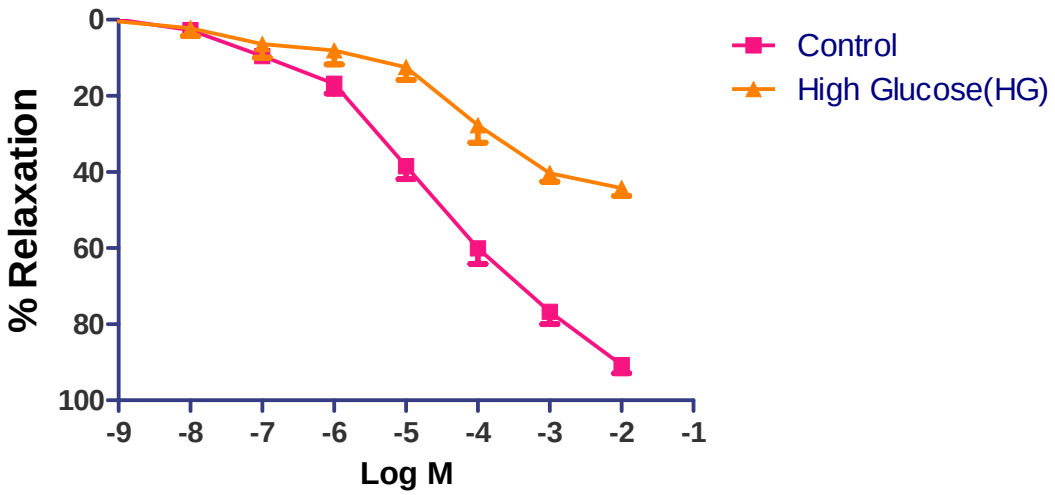

Fig.1.Cumulative dose–response curves to Ach in endothelium-intact aorta rings after incubation with high glucose for 4 h. Data are expressed as mean ± SE. n=6

1.2 Effects of Gallic acid on high glucose-induced acute relaxation dysfunction in aortic rings with intact endothelium

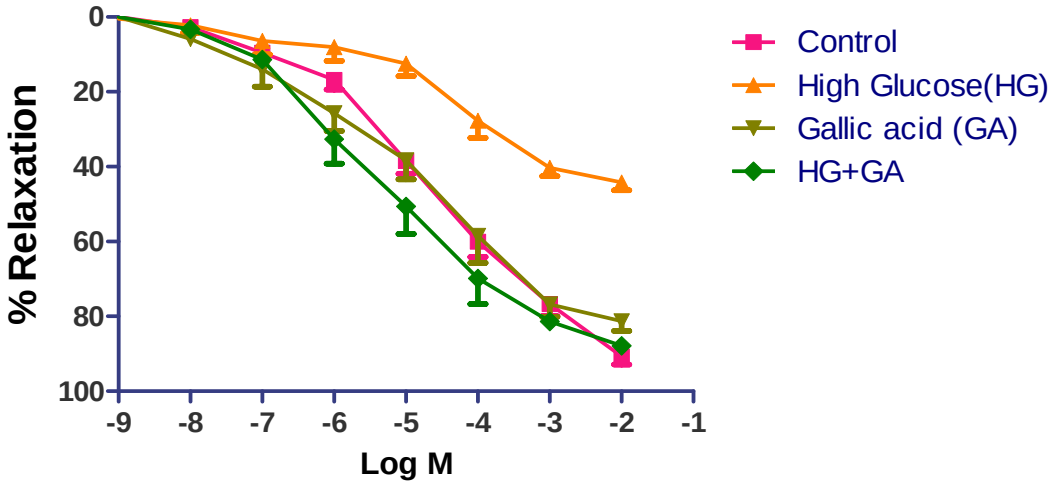

Fig 2.Effect of gallic acid on cumulative dose–response curves to Ach in endothelium-intact aorta rings after incubation with high glucose for 4 h. Data are expressed as mean ± SE. n=6

Table 1:-Compared with control group, incubation with high glucose for 4 h decrease the Ach-induced relaxation.

Group	E_{max}	pD_2
Control	88.69 ± 4.68	4.2 ± 0.2
High Glucose	37.64 ± 5.34[a]	2.9 ± 1.5[a]
Gallic acid	73.41 ± 6.18 [a, b]	3.6 ± 1.4 [a, b]
High glucose + Gallic acid	81.43 ± 2.17 [a, b]	3.9 ± 0.7[a, b]

Values are expressed Mean ± SEM. a =Statistical significant at $P < 0.05$ as compared to control, b = Statistical significant at $P < 0.05$ as compared to High Glucose.

Discussion

Abnormal endothelial function play important role in the pathogenesis of diabetic complications. Because of lack of auto-regulation system for the high glucose transport, intracellular hyperglycaemia could cause serious metabolic dysfunctions both in microvascular and macrovascular damages. [9] Vascular endothelial cell dysfunction in diabetes has been reported associated with hyperglycaemia-induced intra- and extracellular glycation of proteins and to overproduction of glucose elicited oxidants and free radicals.[10] Reduced endothelium-dependent ACh-induced aorta relaxation has been reported in diabetic mice. However, no difference was observed for the endothelium-independent SNP-induced relaxation between diabetic and non-diabetic mice.[10] This is already revealed that 2 h high glucose incubation had no effect of PE-induced contraction, but caused the decreased relaxation in the PE precontracted aortic rings.[11] The previous in vitro study demonstrated that acute exposure of blood vessels to high glucose for more than 3 h showed decreased PE-induced contraction in time-dependent as well as in an endothelium-dependent manner, indicating that acute high glucose exposure could cause both contraction and relaxation dysfunction in an endothelium-dependent way.[8] This could be caused by the glucose-induced impaired endothelial functions. The present in vitro study demonstrated that protective effect of gallic acid on acute exposure of blood vessels to high glucose for 4 h.

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Conclusion

The present investigations concluded that acute high glucose exposures could cause vasodilation dysfunction. Gallic acid could alleviate the high glucose-induced acute endothelium-dependent vascular dysfunction in rat aortic rings.

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