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Effect of quercetin, naringenin & EGCG on α_1 -adrenergic receptor -mediated vascular contraction in pulmonary artery of goat (*Capra hircus*)

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Abstract

Flavonoids have been shown to reduce blood pressure during several pathological conditions. The aim of this work was to investigate the effect of polyphenols such as quercetin, naringenin & EGCG (epigallocatechin-3-gallate) on vasotonic response induced by activation of α_1 -adrenergic receptor in pulmonary artery of *Capra hircus* (PA of *Ch*). The arterial rings prepared from secondary branch of pulmonary artery of *Capra hircus* were mounted in automatic organ bath. Isometric contraction induced by PE (10nM - 100 μ M) under normoxic or hypoxic conditions was recorded in absence or presence of quercetin, naringenin & EGCG. The maximum contractile response (E_{max}) induced by PE in normoxic rings was almost reduced by more than 80% in hypoxic one. The E_{max} obtained from PE-induced contractile response curve in presence of quercetin, naringenin, EGCG was reduced by 77%, 87%, 93% in normoxic rings and by 93%, 95%, 95% in hypoxic rings. In conclusion (i) the PA of *Ch* is sensitive to PE while eliciting contractile response, (ii) the hypoxic state attenuated the α_1 -adrenergic receptor activated contractile response, (iii) the relative inhibitory effect of quercetin, naringenin and EGCG on PE contraction is in the order of EGCG > naringenin > quercetin in normoxic state, (iv) in hypoxic PA rings, the inhibitory effect of quercetin, naringenin and EGCG on PE-induced contraction were almost identical (93-95%). In translating the observation, it is recommended that quercetin, naringenin and EGCG could be useful in decreasing vascular resistance of pulmonary artery thereby controlling the pulmonary hypertension.

Keywords: Quercetin, naringenin, EGCG, pulmonary artery contraction, hypoxia, PE

1. Introduction

Plant compounds such as flavonoids have been reported to exert beneficial effects in cardiovascular diseases, including hypertension. They are able to modulate blood pressure by restoring endothelial function either directly by affecting nitric oxide levels or indirectly through other pathways [1]. Pulmonary arterial hypertension (PAH) is a disease with dysfunction of the endothelin pathway, the prostacyclin pathway and the nitric oxide pathway accompanied by an increase in pulmonary vascular resistance, remodelling, and endothelial dysfunction leading to right ventricular failure and premature death [2]. The available therapies only relieve symptoms and thus slow the progress of the disease but they do not cure PAH [3]. So, there is an urgent need for developing effective therapeutic strategies for treatment of PAH.

Quercetin is found in abundance in onions, apples, broccoli, and berries. Quercetin and some of its chemically synthesized analogs have been reported to produce vasorelaxant action [4]. Quercetin reduces hypertension in rats [5, 6]. The vasorelaxing activity of quercetin is reported in isolated rat aorta [7], rat and porcine vascular smooth muscle [8] and arteries from spontaneously hypertensive rats [9]. Quercetin and its methylated metabolites have more potent endothelium-independent vasodilator effect in resistance than in conductance vessels of rat [10]. Naringenin is a citrus flavonoid found in grapefruit, bitter orange, and other fruits [11]. Naringenin has a relaxant effect on vascular smooth muscle [12, 13] and a regulatory effect on GI functions [14, 15]. The antihypertensive effect of LM-GSPE (Low-molecular-weight procyanidin rich grape seed extract) in SHR (spontaneously hypertensive rats) is endothelium dependent and is mediated by changes in endothelium-derived nitric oxide bioavailability [16]. Naringenin relaxation involve in part the release of nitric oxide and prostaglandins from the endothelium in the isolated rat aorta [12]. Treatment with naringenin shows improvement in diabetic rats [17]. Green tea from the plant *Camellia sinensis* has an active polyphenolic catechin i.e., epigallocatechin-3-gallate (EGCG). The vasodilating effects of EGCG strongly relying on the presence of eNOS and NO production in endothelial cells is seen in isolated aortic rings of endothelial NO knockout mice [18].

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Endothelium/nitric oxide mechanism mediates ECGC-induced vasorelaxation [19, 20, 21, 22]. EGCG stops mammary cancer cell migration by inhibiting NO or NOS and guanylate cyclase [23].

Considering hypoxia as the major reason of pulmonary arterial hypertension in man and animal, the present study investigates the effect of hypoxia on vasotonic response to PE in pulmonary artery of goat (*Capra hircus*) (PA of *Ch*) in absence and presence of quercetin, naringenin and EGCG. The experimental protocol would answer the questions like (i) how does PA of *Ch* responds to contraction induced by PE, (ii) whether quercetin, naringenin and EGCG cause any vasodilatory effect in these α -adrenoceptor-activated contraction, (iii) lastly whether these polyphenols would be useful in ameliorating the altered vasoreactivity of PA of *Ch* under hypoxia or not.

2. Materials and Methods

The whole lungs containing branches of pulmonary artery were obtained from freshly slaughtered goat of local abattoir and transferred in ice cold MKHS to the laboratory. The secondary branches were traced, cleaned and cut into segments of circular rings measuring 1.5-2 mm in length. The arterial rings were employed for isometric contraction studies. Freshly prepared arterial rings were mounted with the isometric force transducer (MLT 0201) positioned on a micro-positioner (Panlab S.I., Spain). Then the arterial rings were equilibrated in MKHS under a resting tension of 1.0 g for a period of 60 min with washing at 15 min interval with MKHS maintained at pH of 7.2-7.4. Following the equilibration period, the vasocontractility was elicited by exposing the arterial rings to ligands. The secondary branch of isolated pulmonary artery of *Capra circus* was mounted in a four

chambered automatic organ bath and exposed to the vasotonic agent PE (10 nM - 100 μ M) in presence of polyphenols like quercetin (10 μ M), naringenin (10 μ M) & EGCG (10 μ M) under normoxic and hypoxic conditions. Separate sets of experiment were conducted for different treatment and control group. The isometric contraction was recorded by personal computer with the help of Lab chart 7 pro software (AD Instrument software, Australia). The tissue holder along with arterial ring was placed in vessel containing 20ml of MKHS (pH 7.2-7.4) maintained at (37.0 \pm 0.5 $^{\circ}$ C) and bubbled with carbogen (95% O₂ +5% CO₂) as normoxic condition and with nominal oxygen (1% O₂ + 4% CO₂ + 95% N₂) simulating hypoxic condition for the hypoxia model. The isometric contraction was recorded by PC with the help of Lab chart 7 pro software (AD Instrument software, Australia). The project was approved by IAEC, C.V. Sc & AH (Regd No.433CPCSEA/CVS/2007) for ex vivo experimentation.

2.1 Statistical Analysis

All values will be expressed as mean \pm standard error of mean (SEM) of measurements in 'n' experiments. The net contraction was expressed as mean gm. The data will be compared using unpaired student's 't' test using Graph Pad Software Quick Calcs. The mean-logEC₅₀ and maximal contraction (E_{max}) was calculated using Graph-Pad Prism 5 software (Graph Pad Prism5, Graph Pad Software Inc., San Diego, CA, U.S.A). A p value < 0.05 and p<0.001 will be considered statistically significant.

3. Results

The chemical structure and natural source of quercetin, naringenin and EGCG have been presented at Figure 1, 2 and 3 respectively.



Fig 1: Quercetin –natural source (apple, broccoli, onion etc.) & structure.

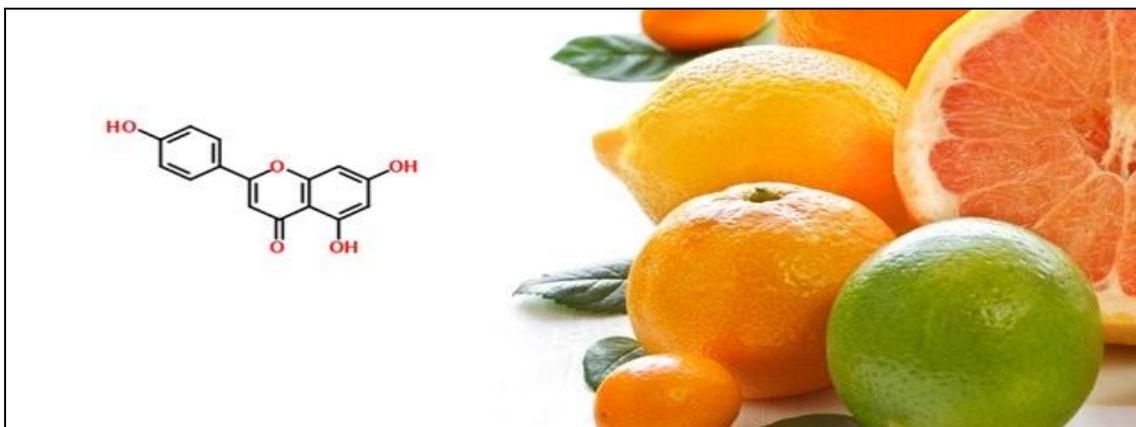


Fig 2: Naringenin – structure & natural source (orange, tangerine etc.)

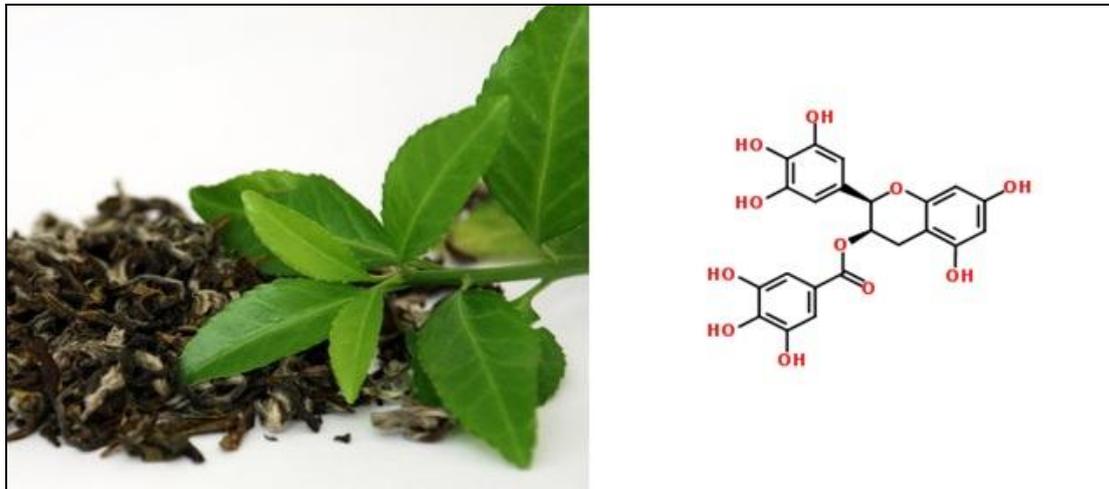


Fig 3: EGCG - natural source (green tea) & structure.

3.1 Effect of quercetin (10 μ M), naringenin (10 μ M) & EGCG (10 μ M) on PE (1nM -100 μ M) concentration response curve elicited in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*.

PE -induced concentration response curve elicited in presence of quercetin (10 μ M) was shifted to right with significant ($p < 0.001$) increase in EC_{50} ($6.43 \pm 0.07 \mu\text{M}$) and with significant ($p < 0.001$) decrease in $E_{B_{\max}}$ ($0.14 \pm 0.00 \text{g}$, $n=6$) in normoxic condition as compared to non-treated normoxic control. Similarly, PE -induced concentration response curve elicited in presence of quercetin was shifted to right with significant ($p < 0.05$) decrease in (EC_{50} $6.46 \pm 0.16 \mu\text{M}$) and significant ($p < 0.001$) decrease in (E_{\max} $0.04 \pm 0.01 \text{g}$) in hypoxic rings in comparison with that of EC_{50} and $E_{B_{\max}}$ treated normoxic one (Table 1, Fig 4).

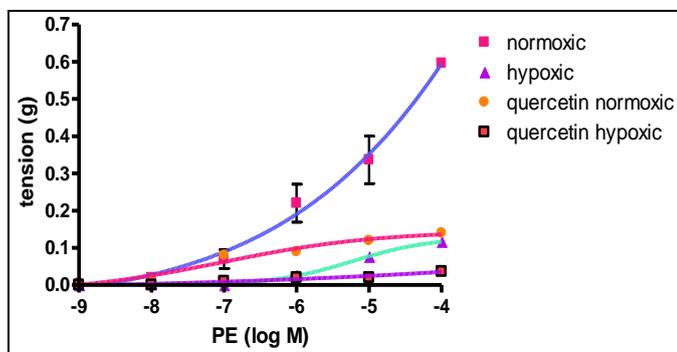


Fig 4: PE (1nM -100 μM) -induced concentration related contractile response in absence (E_{\max}) or in presence ($E_{B_{\max}}$) of quercetin in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*

PE -induced concentration response curve elicited in presence of naringenin (10 μ M) was shifted to right with significant ($p < 0.05$) decrease in EC_{50} ($4.48 \pm 0.13 \mu\text{M}$) and with significant ($p < 0.001$) decrease in $E_{B_{\max}}$ ($0.08 \pm 0.02 \text{g}$) in normoxic condition as compared to non-treated normoxic control. Similarly, PE -induced concentration response curve elicited in presence of naringenin was shifted to right with non-significant ($P < 0.05$) increase in EC_{50} ($4.55 \pm 0.02 \mu\text{M}$) and with significant ($p < 0.001$) decrease in $E_{B_{\max}}$ ($0.03 \pm 0.004 \text{g}$) in hypoxic rings in comparison with that of EC_{50} and $E_{B_{\max}}$ of treated normoxic one (Table. 1, Fig 5).

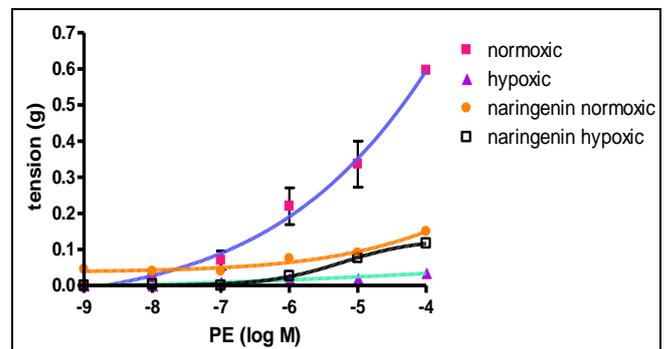


Fig 5: PE (1nM -100 μM) -induced concentration related contractile response in absence (E_{\max}) or in presence ($E_{B_{\max}}$) of naringenin in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*.

PE -induced concentration response curve elicited in presence of EGCG (10 μ M) was shifted to right with significant ($p < 0.05$) increase in EC_{50} ($5.62 \pm 0.1 \mu\text{M}$) and with significant ($p < 0.001$) decrease in $E_{B_{\max}}$ ($0.04 \pm 0.001 \text{g}$, $n=6$) in normoxic condition as compared to non-treated normoxic control. Similarly, PE -induced concentration response curve elicited in presence of EGCG was shifted to right with significant ($p < 0.001$) decrease in EC_{50} ($4.01 \pm 0.02 \mu\text{M}$) and significant ($p < 0.05$) decrease in E_{\max} ($0.03 \pm 0.002 \text{g}$) in hypoxic rings in comparison with that of EC_{50} and $E_{B_{\max}}$ of treated normoxic one (Table 1, Fig 6).

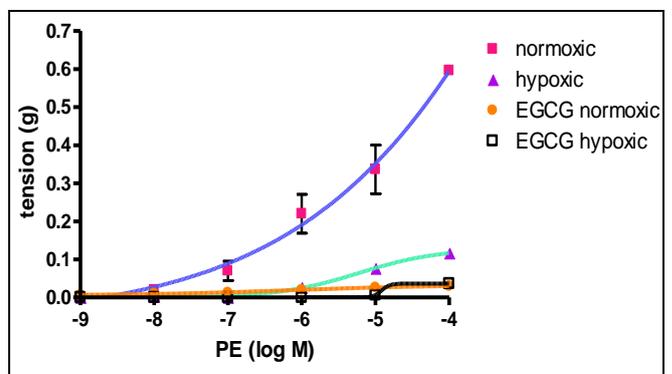


Fig 6: PE (1nM -100 μM) -induced concentration related contractile response in absence (E_{\max}) or in presence ($E_{B_{\max}}$) of EGCG in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*

Table 1: PE (1nM -100µM) -induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of quercetin (10 µM), naringenin (10 µM) & EGCG (10 µM) in normoxic and hypoxic pulmonary arterial rings of *Capra hircus*

treatments	normoxic (N)		hypoxic(H)	
	EC ₅₀	E _{max} /E _{Bmax} (gm)	EC ₅₀	E _{max} /E _{Bmax} (gm)
PE	5.18±0.09	0.60±0.01	5.28±0.03	0.12±0.01 ^c
PE +quercetin	6.43±0.07 ^a	0.14±0.00 ^a	6.46±0.16 ^a	0.04±0.01 ^{bc}
PE + naringenin	4.48±0.13 ^b	0.08±0.02 ^a	4.55±0.02 ^a	0.03±0.004 ^{ad}
PE+ EGCG	5.62±0.10 ^b	0.04±0.001 ^a	4.01±0.02 ^{ac}	0.03±0.002 ^{ad}

a ($p<0.001$) and b ($p<0.05$) represent level of significance between the rows within each column. Data of each row (hypoxic) is compared with the data of normoxic (control) within corresponding column. c ($p<0.001$) and d ($p<0.05$) represent level of significance between the sub-columns (N and H) within each row. Data of each 'H' column in a particular row is compared with the corresponding data of 'N' column.

4. Discussion

In PA of *Ch* we observed that (i) the maximal contractile tension to phenylephrine was about 0.6g and this indicates that α_1 -adrenergic receptor stimulation moderately increases vascular resistance, (ii) naringenin, quercetin and EGCG significantly reduced the PE-induced contraction clearly demonstrates that these polyphenols could be activating vasorelaxation pathways or antagonising the α_1 -adrenergic receptor mediated contraction, (iii) experimental hypoxia significantly reduced the α_1 -adrenergic receptor mediated contraction and these three polyphenols further reduced the PE-induced contraction which indicates that these polyphenols have little therapeutic use in hypoxic-induced vasospasm.

Phenylephrine, an adrenergic receptor agonist mediates vasoconstriction differentially in both normoxic & hypoxic goat pulmonary arteries. A greater and low contractile response to PE in normoxic and hypoxic artery clearly indicate that α_1 -receptor sensitivity to PE is reduced with decrease in oxygen tension in bathing solution. Quercetin inhibited the PE mediated contractile response in normoxic rings with decrease in EC₅₀ by 1.25 log units and maximal response by 77%. Similarly, in the presence of quercetin, PE dose response curve was shifted to right with increase in EC₅₀ by 1.28 log unit and decrease in maximal response by 93% in hypoxic rings. Decrease in EC₅₀ of PE by quercetin observed in both normoxic & hypoxic rings might be due to modulation of binding of α_1 -adrenergic receptor to PE that does not appear to enhance the PE-mediated contractile response. In the descending thoracic aorta of Male Sprague-Dawley rats PE-induced contractile response has been reported to be inhibited by quercetin and this is resulted from activation of endothelium-dependent relaxation [24]. Quercetin induced decrease in vasotonic response to PE has been reported in diabetic rat aortic ring [12]. Quercetin increases the activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione levels which decreases serum levels of malondialdehyde. These results suggest that quercetin effects on neuronal function and metabolism could be due to potential protective antioxidants against hypobaric hypoxia-induced lesions [25]. This reduction of PE-induced vasotonic response by quercetin observed in normoxic and hypoxic artery could be due to modulation of signal transduction mechanism involving the stimulation of α_1 -adrenergic receptor in goat superior mesenteric artery [26] or activation of endothelial NO pathways that cause vasorelaxation as reported in descending thoracic aorta of Male Sprague-

Dawley rats [24] or reduction of intracellular Ca²⁺ that cause decreased vasotonic response in pre-contracted aortic rings isolated from euglycemic rats [12] and diabetic rats [27].

Naringenin inhibited PE-mediated contractile response with reduction of EC₅₀ by 0.7 log unit and E_{Bmax} by 87% in normoxic and EC₅₀ by 0.63 log unit and E_{Bmax} by 95% in hypoxic pulmonary arterial rings of *Capra hircus* clearly demonstrate that naringenin also causes vasodilation. The maximum contractile response of endothelium-intact rings to PE was significantly lower in naringenin-treated diabetic rats as compared to untreated diabetic rats [28]. The subchronic administration of naringenin for 6 weeks could exert an anti-hyperglycemic effect and lowers contractile responsiveness of thoracic aorta rings to phenylephrine [29]. Our present observation is in close agreement with their observations that naringenin significantly reduced the PE induced maximal contractile response in normoxic condition. The possible mechanisms for reduction of PE induced maximal contractile response by naringenin can be either enhanced activity of EDRF (NO) or EDHF or reduced intracellular [Ca²⁺]_i in response to adrenergic receptor signalling through which naringenin improved aortic reactivity in streptozotocin-diabetic rats [17].

In presence of EGCG, the vasotonic effect of α_1 -adrenergic receptor stimulation was significantly reduced in both normoxic and hypoxic pulmonary arterial rings of *Capra hircus*. PE induced concentration response was inhibited in presence of EGCG with increase in EC₅₀ by 0.44 log unit and maximal response by 93% in normoxic pulmonary arterial rings of *Capra hircus*. In hypoxic rings, EGCG inhibited PE induced vasotonic response with decrease in EC₅₀ by 1.17 log unit and E_{Bmax} by 95%. The inhibitory effect of EGCG in both normoxic and hypoxic rings are almost identical. EGCG increased endothelial nitric oxide (eNOS) activity dose-dependently. Our observation for potent vasodilatory effect of EGCG in both normoxic and hypoxic pulmonary arterial rings of *Capra hircus* could be attributed to the mechanisms involving endothelium-dependent activation of eNOS as in bovine aortic endothelial cells where EGCG activates endothelial nitric oxide synthase by a phosphatidylinositol-3-OH-kinase-, cAMP-dependent protein kinase-, and Akt dependent pathway and leads to endothelial-dependent vasorelaxation. [19].

The vasorelaxation of quercetin, naringenin & EGCG in PE-precontracted rings is more in hypoxic than normoxic rings. Our observations clearly demonstrated that polyphenols exhibited significant inhibitory effect on PE induced vasotonic response in pulmonary arterial rings of *Capra hircus* in the of EGCG> naringenin>quercetin in normoxic and EGCG= naringenin > quercetin in hypoxic condition.

5. Conclusion

Quercetin, naringenin & EGCG potentially attenuated the α_1 -adrenergic receptor mediated vasotonic response in both normoxic & hypoxic pulmonary arterial rings of *Capra hircus*. Vasorelaxation to these polyphenols could be

mediated via direct activation of endothelial NO that increases cGMP to produce vascular smooth muscle relaxation. Another reason could be due to interference in Ca²⁺ entry pathways or modulation of α_1 -adrenergic receptor couples signalling pathways. Hence, the use of these polyphenols could be effective in reducing increased vascular resistance or hypertension arising from inflammatory damage in PAH with prevailing hypoxic condition by improving blood circulation of lungs.

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7. Conflict of interest

We declare we have no conflict of interest.

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