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Original article

Effects of quercetin, naringenin and EGCG on 5HT and histamine induced contraction in pulmonary artery and its modulation by hypoxia

Santwana Palai, Kautuk K. Sardar, Prakash C. Behera* and Subash C. Parija

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India

**Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India*

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Abstract

The importance and role of phytochemicals in promoting good health by its antioxidant effect is well documented. The aim of this work was to investigate the effect of three defined polyphenols such as quercetin, naringenin and EGCG on vasotonic response induced by activation of serotonin and histamine receptor in pulmonary artery of *C. hircus* (PA of *Ch*). The arterial rings prepared from secondary branch of pulmonary artery of *C. hircus* was mounted in automatic organ bath. Isometric contraction induced by 5HT and histamine (1nM-100µM) under normoxic or hypoxic conditions was recorded in absence or presence of quercetin, naringenin and EGCG. The maximum contractile response (E_{max}) induced by 5HT and histamine in normoxic rings was almost reduced by more than 50% in hypoxic one. The E_{max} obtained from 5HT induced contractile response curve was greater than histamine in both normoxic and hypoxic rings. The E_{max} obtained from 5HT-induced contractile response curve in presence of quercetin, naringenin, EGCG was reduced by 44%, 63%, 38%, in normoxic rings and by 85%, 93%, 89% in hypoxic rings,respectively Similarly, the E_{max} obtained from histamine-induced contractile response curve in presence of quercetin, naringenin, EGCG was reduced by 36%, 51%, 60% in normoxic rings and by 89%, 83%, 84%, respectively in hypoxic rings. In conclusion, (i) the PA of *Ch* is more sensitive to 5HT than histamine while eliciting contractile response, (ii) the hypoxic state attenuated the 5HT and histamine receptor activated contractile response, (iii) the relative inhibitory effect of quercetin, naringenin and EGCG on 5HT and histamine-induced contraction is in the order of naringenin> quercetin> EGCG and EGCG> naringenic> quercetin, respectively, in normoxic state, (iv) in hypoxic PA rings the inhibitory effect of quercetin, naringenin and EGCG on 5HT and histamine-induced contraction were almost identical (83-93%). In translating the observation, it is recommended that quercetin, naringenin and EGCG could be useful in decreasing vascular resistance of pulmonary artery and thereby controlling the pulmonary hypertension.

Key words: Quercetin, naringenin, EGCG, pulmonary artery contraction, hypoxia

1. Introduction

Pulmonary arterial hypertension (PAH) is a disease of increase in pulmonary vascular resistance and remodelling involving dysfunction of the endothelin, prostacyclin pathway and nitric oxide pathways, leading to right ventricular failure and premature death (Boucherat *et al*., 2015). The available therapies (phosphodiesterase type 5 inhibitors, endothelin-1 receptor antagonists or prostacyclin mimetics) relieve symptoms and slow the progress of the disease but has no certain cure (Sitbon *et al*., 2014). Thus, there is a necessity for novel effective therapeutic strategies for PAH.

Quercetin [2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one], a flavonoid present in onions, apples, peppers, tomatoes, cruciferous vegetables including broccoli, cabbage and sprouts (Hertog *et al.*,1993) is an antioxidant (Morel *et al*., 1993), antimutagenic (Harwood *et al*., 2007), anti-inflammatory (Rogelio *et al*., 2007), leishmanicidal (Marín *et al*., 2009), antihypertensive (Duarte *et al*., 2001; Yamamoto and Oue, 2006) , iron chelator (Ferrali *et al*., 1997) and vasorelaxant (Chen and Pace-Asciak, 1996, Chen *et al*., 2004). Naringenin [(2S)-5,7-Dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4H-chromen-4-one], a citrus flavonoid found in grapefruit, bitter orange and other fruits (Liu *et al*., 2016) has anti-inflammatory (Tsai *et al*., 2012), antimutagenic (Shi *et al*., 2009), antioxidant (Mershiba *et al*., 2013), anticancerous (Abaza *et al*., 2015), antiatherogenic (Lee *et al*., 2003), vasorelaxant (Ajay *et al*., 2003) with vasoactivity (Saponara *et al*., 2006) and GI regulator (Yang *et al*., 2014; Sanders *et al*., 2014) properties. Epigallocatechin-3-gallate (EGCG) [(2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate], a catechin from green tea (*Camellia sinensis)* leaves is a poteintial source of antioxidant (Guo *et al*., 2005; Weinreb

Author for correspondence: Dr. Subash C. Parija
Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Orissa University of Agriculture and Technology, Bhubaneswar-751003, Odisha, India
E-mail: profscparijaouat4691@gmail.com
Tel.: +91-9437356387

et al., 2004), radical scavenger (Weinreb *et al*., 2004), metal chelator, anti-carcinogen (Lambert and Elias, 2010), anti-apoptotic (Nie *et al*., 2002) and anti-inflammatory (Singh *et al*., 2010).

Table 1: 5HT (1nM -100μM) and histamine (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 *C. hircus*

	Normoxic (N)		Hypoxic(H)	
treatment groups	**EC_{50}**	**E_{max}/E_{Bmax} (gm)**	**EC_{50}**	**E_{max}/E_{Bmax} (gm)**
5HT	**5.77±0.01**	**1.34±0.16**	**4.71±0.01^{c}**	**0.50±0.004^{c}**
5HT +Quer	**5.64±0.02 ^{a}**	**0.76±0.06 ^{a}**	**3.46±0.50 ^{ad}**	**0.20±0.03 ^{ac}**
5HT + Nari	**5.45±0.02 ^{a}**	**0.50±0.03 ^{a}**	**4.81±0.003^{ac}**	**0.10±0.02 ^{ac}**
5HT+ EGCG	**4.66±0.001 ^{a}**	**0.83±0.04 ^{a}**	**5.11±0.002 ^{ac}**	**0.41±0.04 ^{ac}**
Histamine	**5.01±0.01**	**1.22±0.06**	**4.85±0.01^{c}**	**0.45±0.05 ^{c}**
Hist + Quer	**5.40±0.004 ^{a}**	**0.78±0.02 ^{a}**	**4.62±0.02 ^{ac}**	**0.14±0.01 ^{ac}**
Hist + Nari	**5.29±0.03 ^{a}**	**0.60±0.05 ^{a}**	**5.13 ±0.06 ^{ad}**	**0.21±0.004 ^{ac}**
Hist+ EGCG	**5.35±0.04 ^{a}**	**0.49±0.04 ^{a}**	**4.99±0.01 ^{ac}**	**0.20±0.01 ^{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.

The effect of quercetin is partly endothelium-dependent (Ajay *et al*., 2003; Khoo *et al*., 2010) involving nitric oxide in rat isolated thoracic aorta (Chen and Pace-Asciak, 1996 ; Chan *et al*., 2000 ; Ajay *et al*., 2003) and endothelium-independent in isolated rat vascular smooth muscle (Duarte *et al*.,1993); isolated rat thoracic and abdominal aorta, isolated iliac arteries and mesenteric resistance vascular bed (Perez-Vizcaino *et al*.,2002) and a combination of these actions in rat aorta ring preparations and single tail artery myocytes (Fusi *et al*., 2003).

Naringenin has a relaxant effect on vascular smooth muscle of rat thoracic aorta (Ajay *et al*., 2003); rat and bovine aorta (Orallo *et al*., 2005), rat thoracic aortic rings (Saponara *et al*., 2006); mouse isolated stomach (Amira *et al*., 2008); rat colonic smooth muscle (Yang *et al*., 2014); interstitial cells of Cajal from murine small intestine (Kim & Kim, 2017) and protects diabetic rats (Fallahi *et al*., 2012).

The vasodilating effects of EGCG rely on eNOS and NO production in endothelial cells in isolated aortic rings of endothelial NO knockout mice (Lorenz *et al*., 2015), rat aorta (Alvarez *et al*., 2006); improve cardiovascular and metabolic function in spontaneously hypertensive rats (Potenza *et al*., 2007) ; porcine coronary artery rings (Auger *et al*., 2010), rat thoracic aorta (Aggio *et al*., 2013); mesenteric vascular beds from WKY rats (Kim *et al*., 2007); endothelial cells of bovine (*Lorenz et al., 2004*). The chemical structure and natural source of quercetin, naringenin and EGCG is given in (Figures 1A, 1B and 1C), respectively.

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 5HT and histamine in pulmonary artery of goat (*C. 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 5HT and histamine, (ii) whether quercetin, naringenin and EGCG cause any vasodilatory effect in these serotonergic and histaminergic receptor-activated contraction, (iii) whether goat pulmonary artery could be employed as model for study for vasorelaxation under hypoxia and (iv) Lastly, if these polyphenols would be useful in ameliorating the altered vasoreactivity of PA of *Ch* under hypoxia or not.

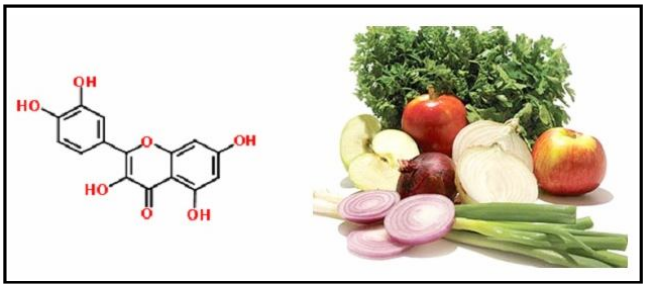


Figure 1A: Chemical structure of quercetin and natural sources- onions, apples, tomatoes, cruciferous vegetables.

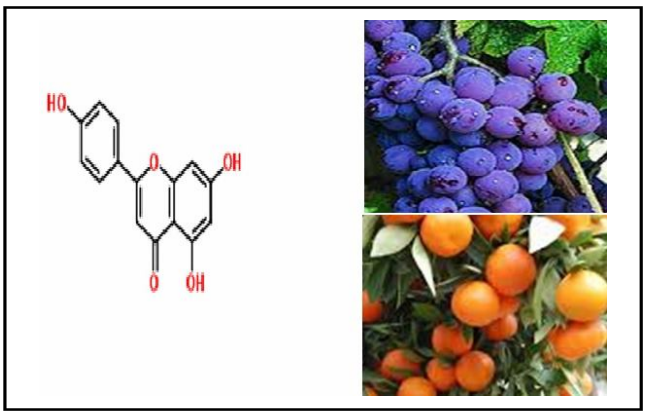


Figure 1B: Chemical structure of naringenin and natural sources- grapes, orange

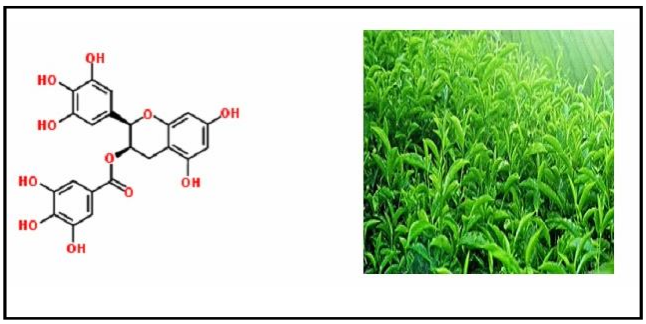


Figure 1C : Chemical structure of ECGC and natural source- green tea.

2. Materials and Methods

The whole lungs containing branches of pulmonary artery obtained from freshly slaughtered goat of local abattoir was transferred in ice cold Modified Krebs-Henseleit Solution (MKHS) to the laboratory. The arteries were cleared of connective tissues, fascia then cut into segments of circular rings measuring 1.5-2 mm in length and 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 *C. hircus* was mounted in a four chambered automatic organ bath and exposed to vasotonic agent like 5HT (10 nM-100 µM) and histamine (10 nM-100 µM) in presence of polyphenols like quercetin (10 µM), naringenin (10 µM) and EGCG (10 µM) under normoxic and hypoxic conditions. Separate sets of experiments were conducted for different treatment and control groups. 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 20 ml of MKHS (pH 7.2-7.4) maintained at (37.0±0.5^{0}C) and bubbled with carbogen (95% O_2 +5% CO_2) as normoxic condition and with nominal oxygen (1% O_2 + 4% CO_2 + 95% N_2) 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). All the experiments were carried after approval from IAEC, C.V.Sc and AH (Regd No.433CPCSEA/CVS/2007)

2.1 Statistical analysis

All values are expressed as mean ± Standard error of mean (SEM) of measurements in 'n' experiments. The net contraction was expressed as mean gm. The data was compared using unpaired student's 't' test using GraphPad Software Quick Calcs. The mean-logEC_{50} and maximal contraction (E_{max}) was calculated using Graph-Pad Prism 5 software (GraphPad Prism5, GraphPad Software Inc., San Diego, CA, U.S.A). A '*p*' value < 0.05 and *p* < 0.001 was considered statistically significant.

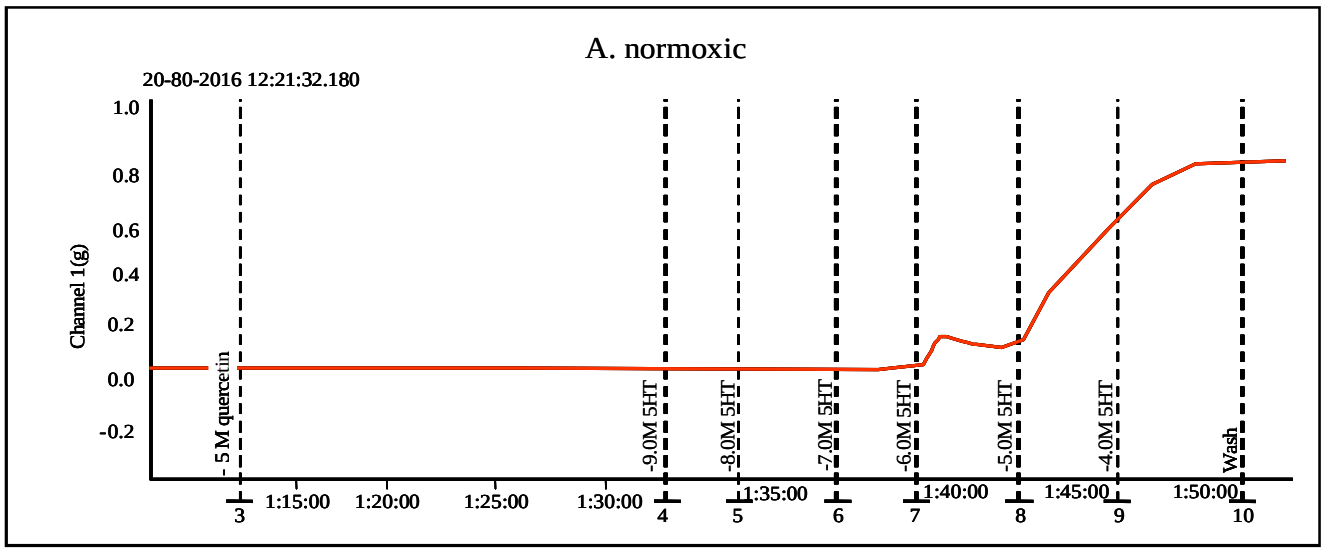
3. Results

Effect of quercetin (10 µM), naringenin (10 µM) and EGCG (10 µM) on 5HT (1 nM -100 µM) concentration related contractile response elicited in normoxic and hypoxic pulmonary arterial rings.

5HT- induced CRC response curve elicited in presence of quercetin (10 µM) was shifted to right with significant (*p* < 0.001) decrease in EC_{50} and E_{Bmax} (5.64 ± 0.02 and 0.76 ± 0.06 g, n = 6) in normoxic condition as compared non-treated normoxic control (EC_{50} 5.77 ± 0.01, E_{max} 1.34 ± 0.16 g, n = 6). Similarly, in presence of quercetin 5HT-induced CRC response curve was shifted to right with significant (*p* < 0.05, *p* < 0.001) decrease in EC_{50} and E_{Bmax} (3.46 ± 0.5, 0.2 ± 0.03 g, n = 6) in hypoxic rings as compared to that of EC_{50} and E_{Bmax} of treated normoxic group (Figures 2A, B, C).

In presence of naringenin (10 µM), 5HT-induced CRC response curve was shifted to right with significant (*p* < 0.001) decrease in EC_{50} and E_{Bmax} (5.45 ± 0.02 0.5 ± 0.03 g, n = 6) in normoxic condition as compared to non-treated normoxic control (EC_{50} 5.77 ± 0.01, E_{max} 1.34 ± 0.16 g, n = 6). Similarly, 5HT-induced CRC response curve elicited in presence of naringenin was shifted to right with significant (*p* < 0.001) decrease in EC_{50} and E_{Bmax} (4.81 ± 0.003 µM, 0.10 ± 0.02 g, n = 6) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax} of treated normoxic group (Figures 3A, B, C).

CRC response curve elicited by 5HT in presence of EGCG (10 µM) was shifted to right with significant (*p* < 0.001) decrease in EC_{50} and E_{Bmax} (4.66 ± 0.001 µM, 0.83 ± 0.04 g, n = 6) in normoxic condition as compared to non-treated normoxic control (EC_{50} 5.77 ± 0.01, E_{max} 1.34 ± 0.16 g, n = 6). Similarly, 5HT induced CRC response curve elicited in presence of EGCG was shifted to right with significant (*p* < 0.001) increase in (EC_{50} 5.11 ± 0.002 µM) and significant (*p* < 0.001) decrease in (E_{max} 0.41 ± 0.04 g, n = 6) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax} of treated normoxic group (Figures, 4 A, B, C).



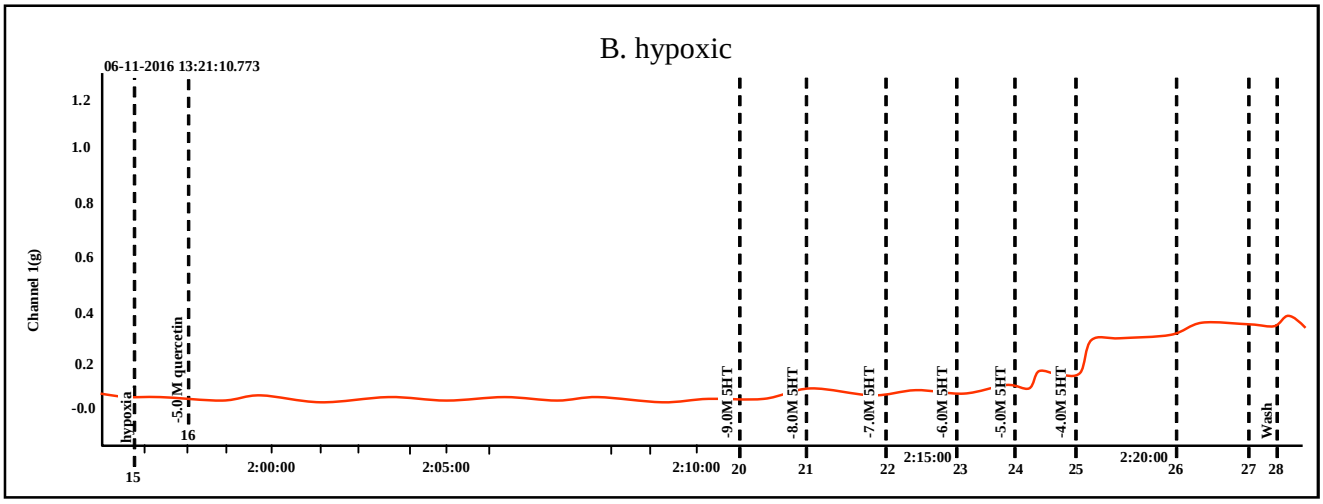


Figure 2: Representative raw trace showing effect of 5HT (1 nM-100 µM) induced concentration related contractile response in presence of quercetin(10 µM) in normoxic and hypoxic rings.

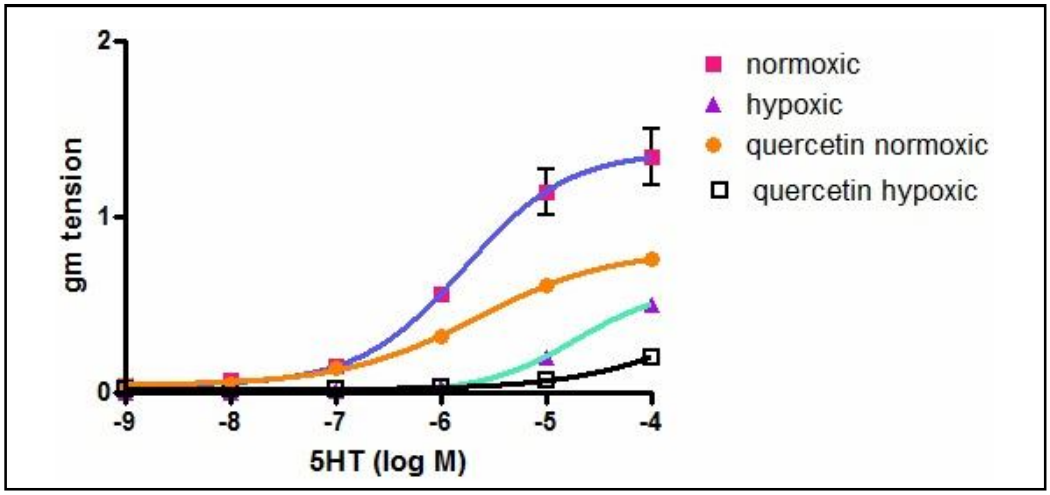
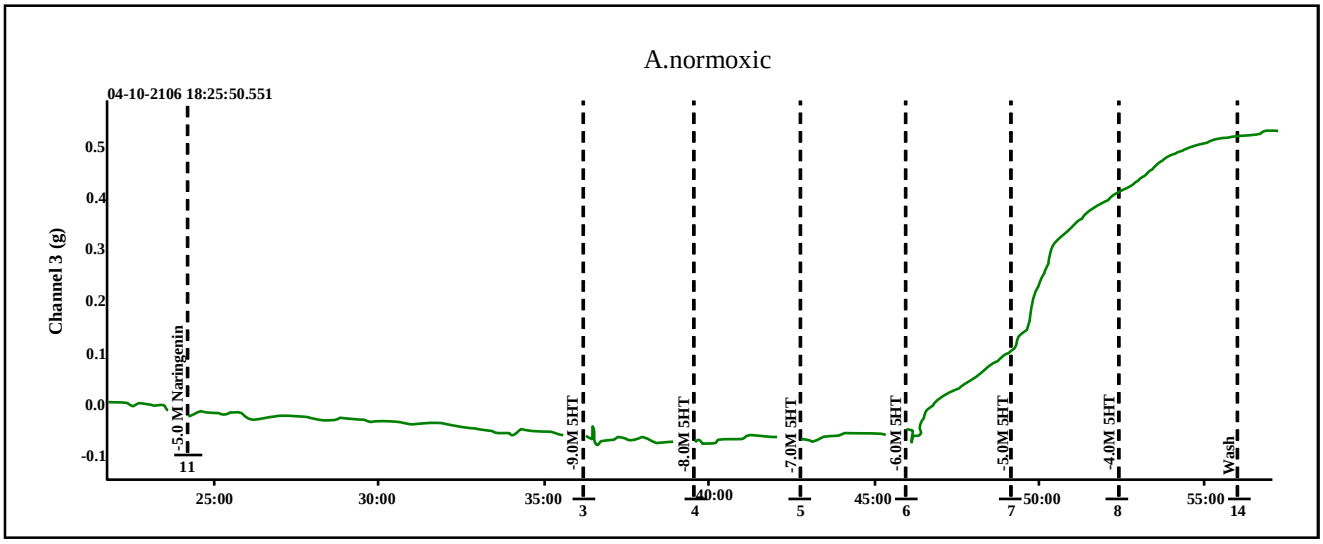


Figure 2C: 5HT (1nM-100 µM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of quercetin in normoxic and hypoxic pulmonary arterial rings of *C. hircus*.



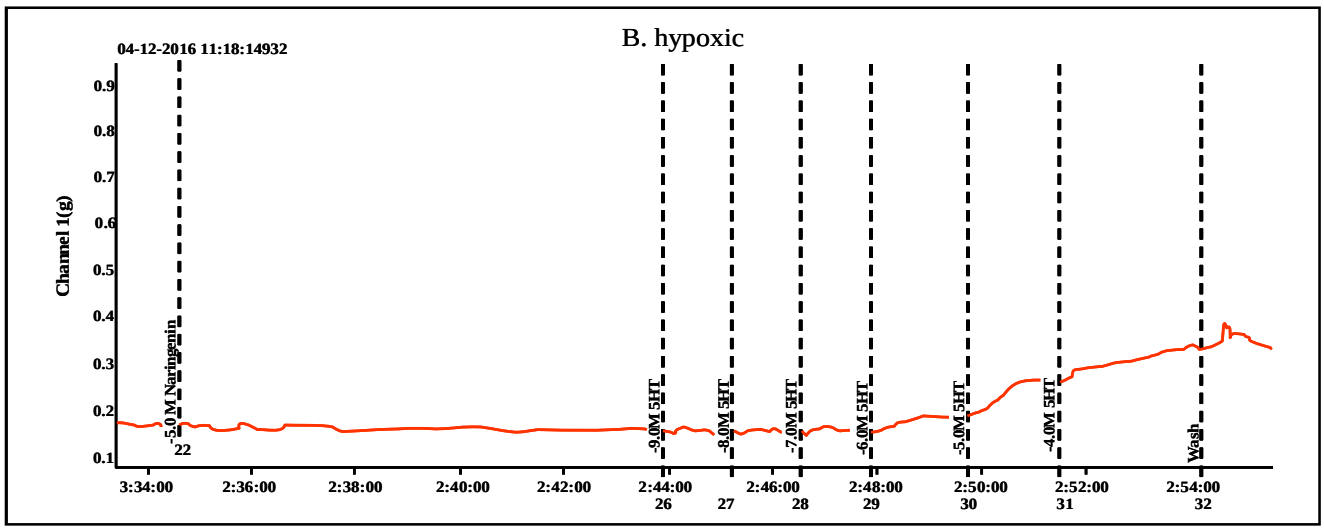


Figure 3: Representative raw trace showing effect of 5HT (1 nM-100 µM) induced concentration related contractile response in presence of naringenin (10 µM) in normoxic and hypoxic rings.

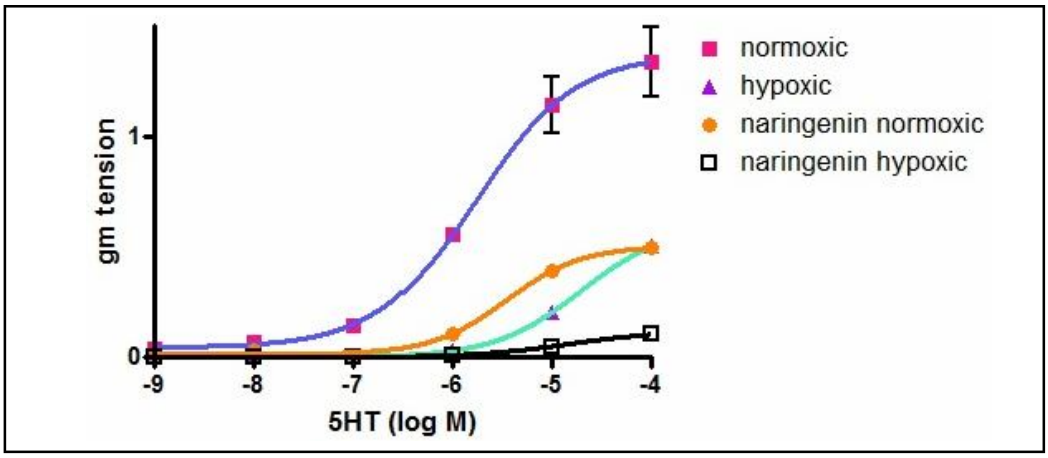
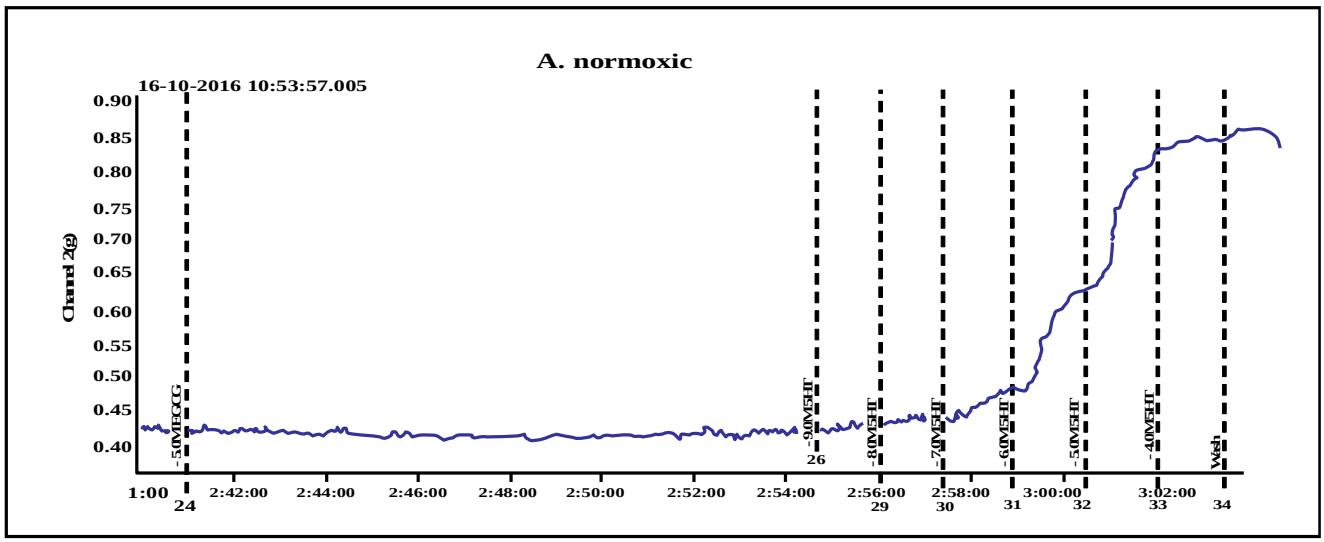


Figure 3C: 5HT (1 nM-100 µM) induced concentration related con-tractile response in absence (E_{max}) or in presence (E_{Bmax}) of naringenin in normoxic and hypoxic pulmonary arterial rings of *C. hircus*.



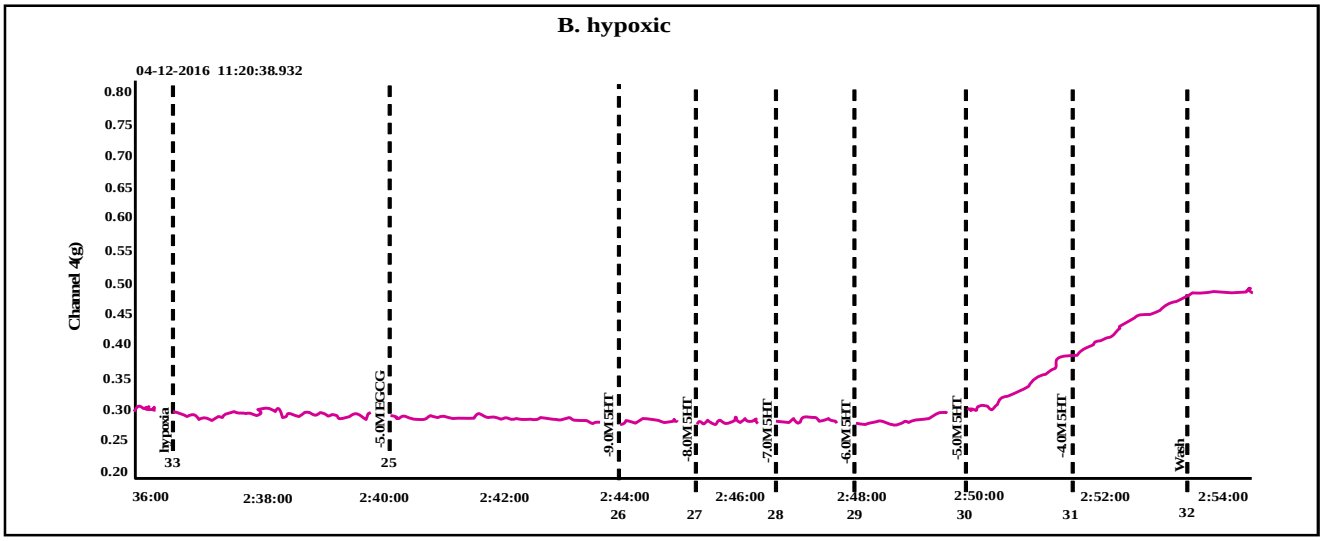


Figure 4: Representative raw trace showing effect of 5HT (1 nM-100 µM) induced concentration related contractile response in presence of EGCG (10 µM) in normoxic and hypoxic rings.

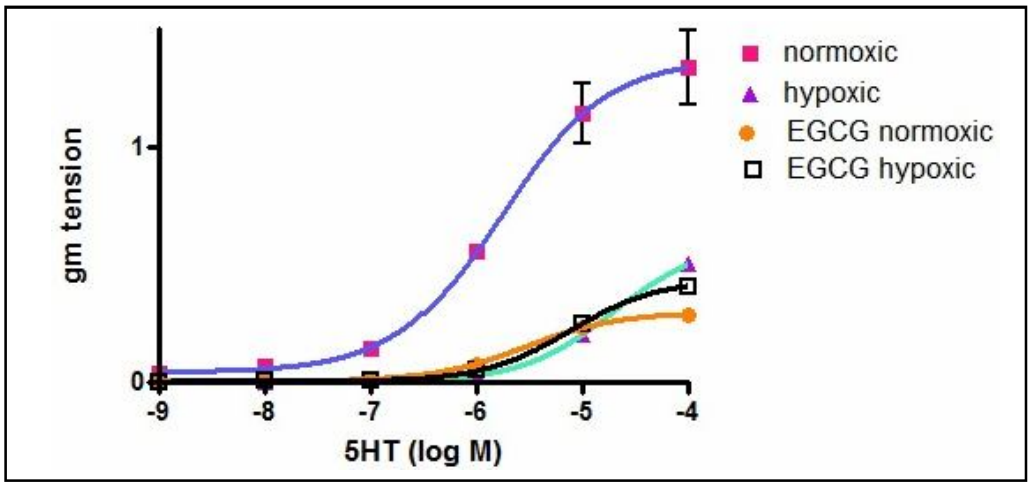
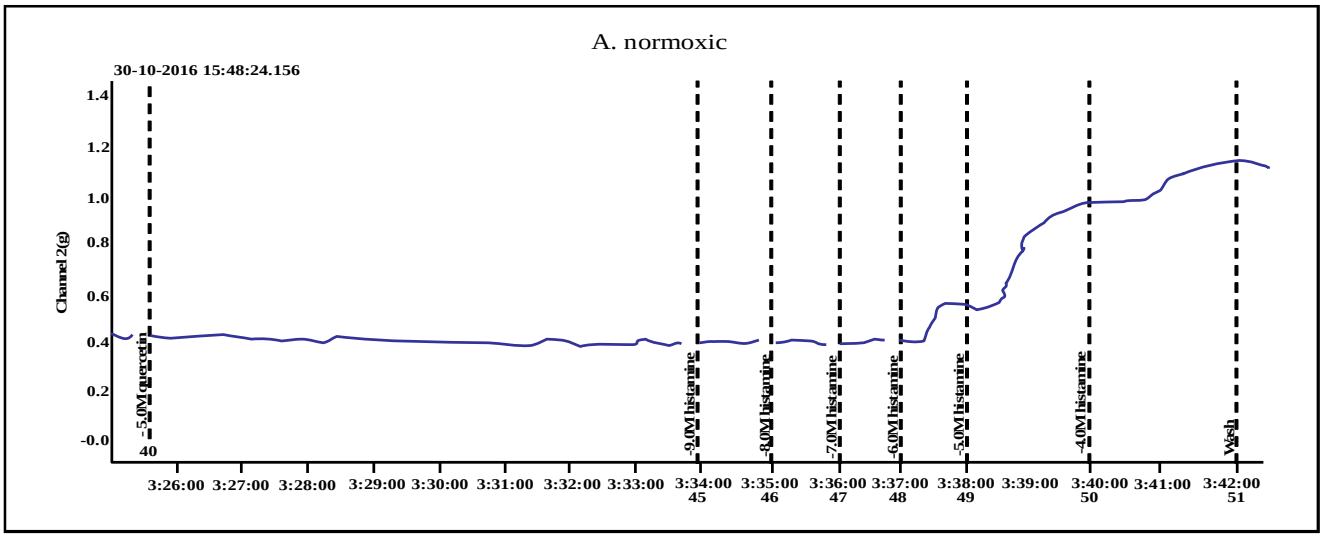


Figure 4C: 5HT (1nM-100 µM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of EGCG in normoxic and hypoxic pulmonary arterial rings of *C. hircus*.



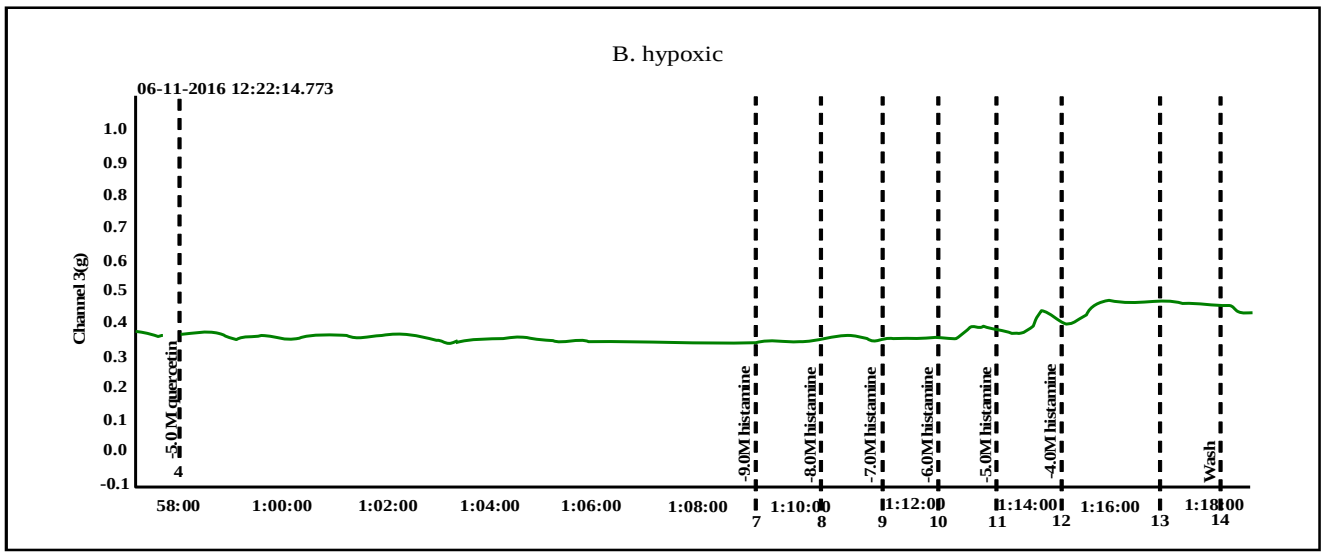


Figure 5: Representative raw trace showing effect of histamine (1nM-100 µM) induced concentration related contractile response in presence of quercetin(10 µM) in normoxic and hypoxic rings.

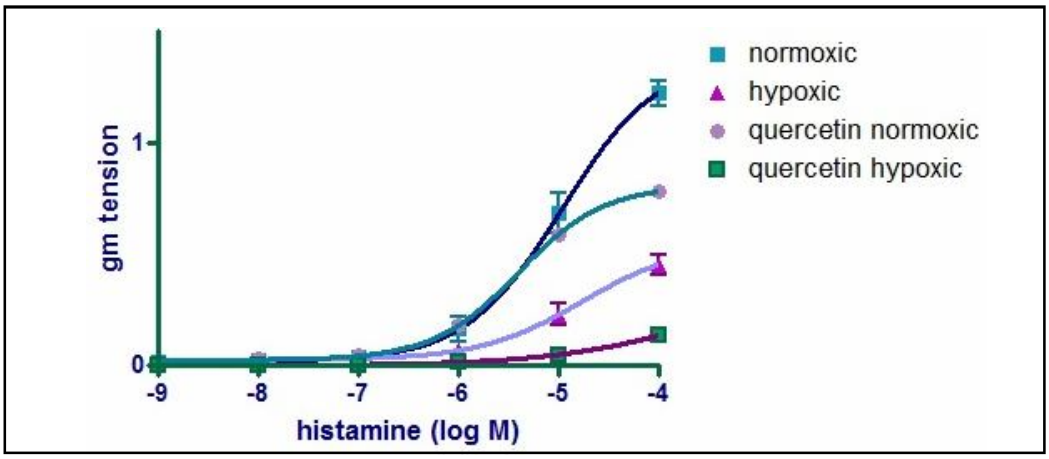
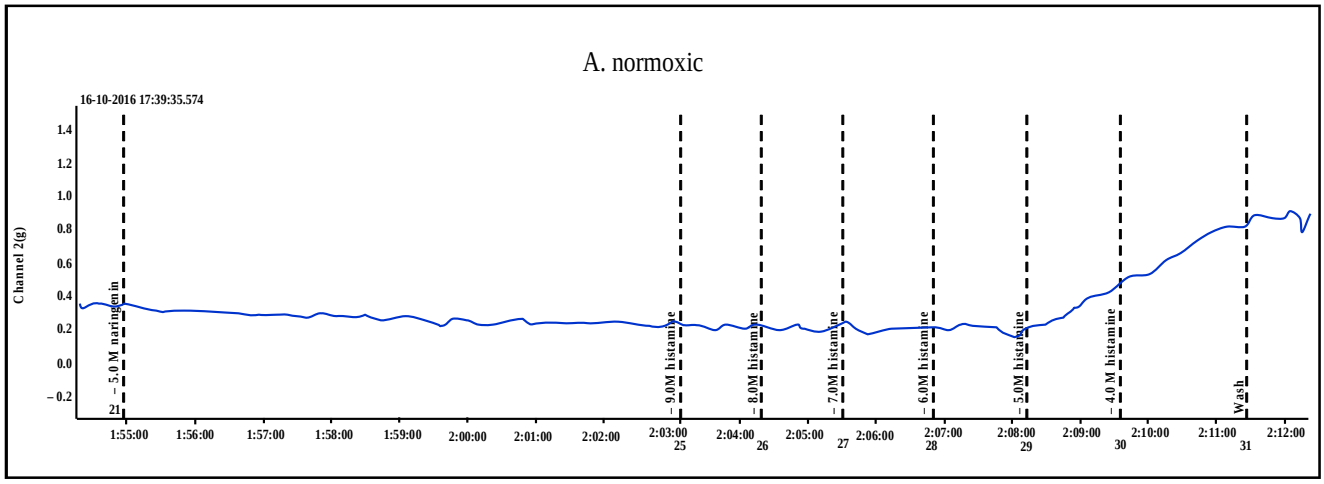


Figure 5C: Histamine (1nM-100 µM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of quercetin in Normoxic and Hypoxic pulmonary arterial rings of *C. hircus*.



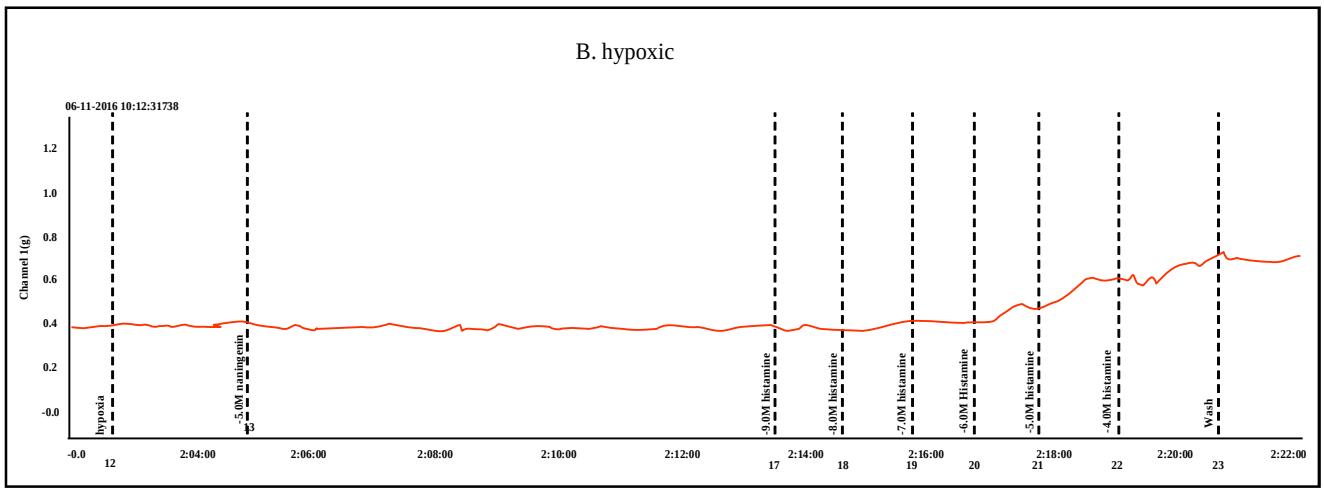


Figure 6: Representative raw trace showing effect of histamine (1 nM-100 µM) induced concentration related contractile response in presence of naringenin (10 µM) in normoxic and hypoxic rings.

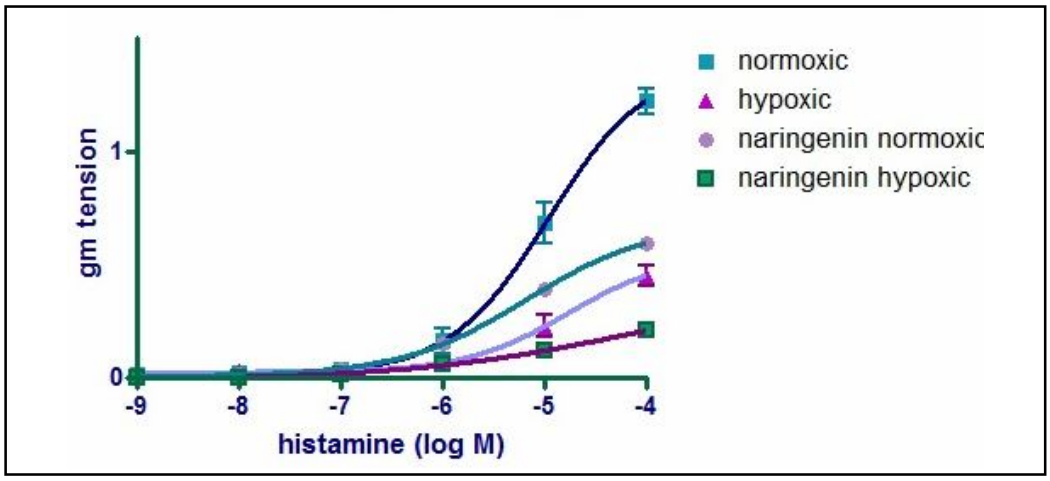
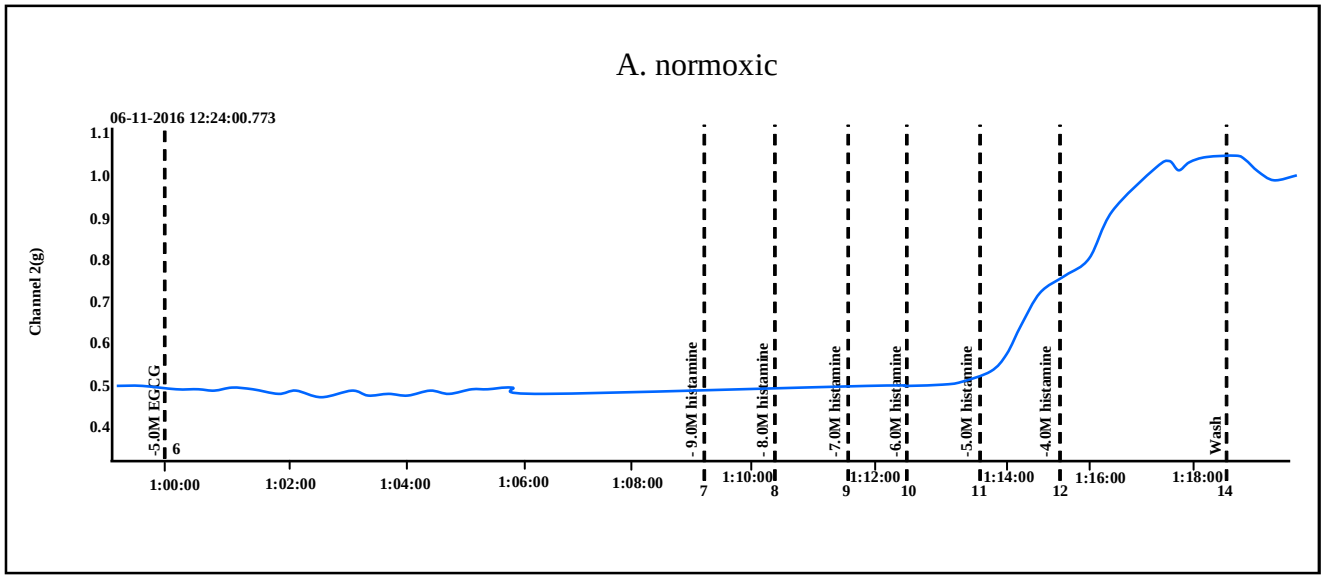


Figure 6C: Histamine (1nM-100 µM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of naringenin in normoxic and hypoxic pulmonary arterial rings of *C. hircus*.



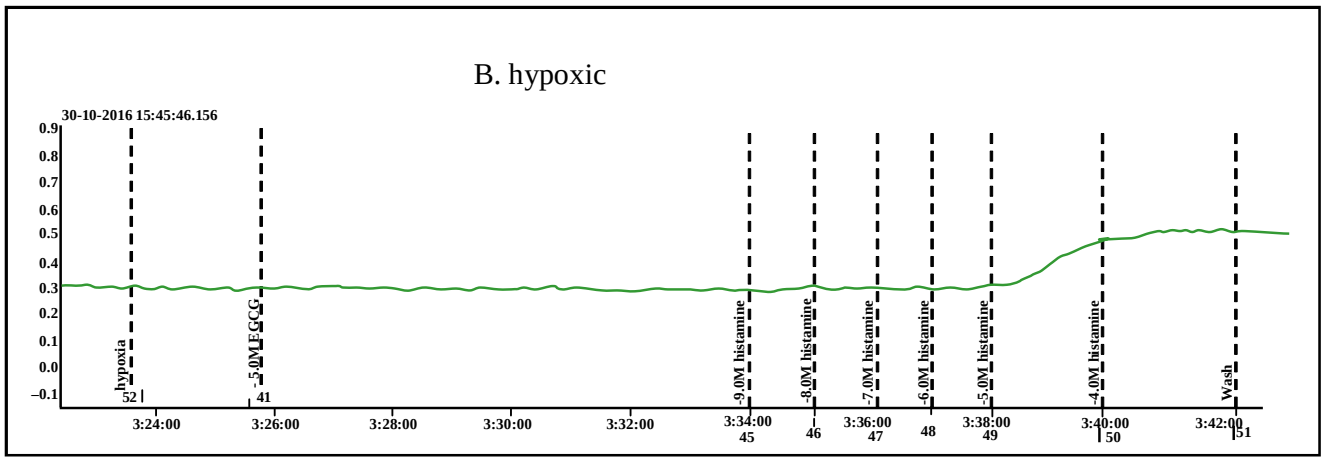


Figure 7: Representative raw trace showing effect of histamine (1nM-100 μM) induced concentration related contractile response in presence of EGCG (10 μM) in normoxic and hypoxic rings

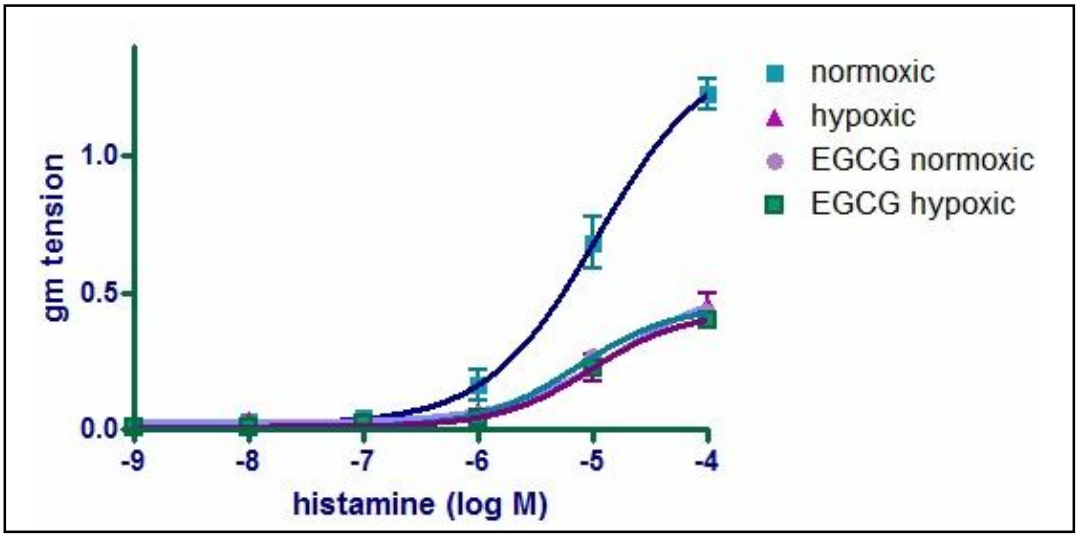


Figure 7C: Histamine (1 nM-100 μM) induced concentration related contractile response in absence (E_{max}) or in presence (E_{Bmax}) of EGCG in normoxic and hypoxic pulmonary arterial rings of *C. hircus.*

Effect of quercetin (10 μM), naringenin (10 μM) and EGCG (10 μM) on histamine (1 nM-100 μM) concentration related contractile response elicited in normoxic and hypoxic pulmonary arterial rings.

Histamine-induced CRC response curve elicited in presence of quercetin (10 μM) was shifted to right with significant ($p < 0.001$) increase in EC_{50} (5.40 ± 0.004 μM) and with significant ($p < 0.001$) decrease in E_{Bmax}(0.78 ± 0.02 gms, n = 6) in normoxic condition as compared to nontreated normoxic control (EC_{50} 5.01 ± 0.01 μM, E_{max} 1.22 ± 0.06 g; n = 6). Similarly, histamine induced CRC response curve elicited in presence of quercetin was shifted to right with significant ($p < 0.001$) decrease in (EC_{50} 4.62 ± 0.02 μM, E_{max} 0.14 ± 0.01 g, n = 6) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax} normoxic group (Figures 5A, B, C).

In presence of naringenin (10 μM), histamine induced CRC response curve was shifted to right with significant ($p < 0.001$) increase in EC_{50} (5.29 ± 0.03 μM) and with significant ($p < 0.001$) decrease in E_{Bmax} (0.60 ± 0.05 g, n = 6), in normoxic condition as compared to non-treated normoxic control (EC_{50} 5.01 ± 0.01 μM, E_{max} 1. 22 ± 0.06 g; n = 6). Similarly, histamine induced CRC response curve elicited in presence of naringenin was shifted to right with significant ($p < 0.05$) decrease in (EC_{50} 5.13 ± 0.06 μM) with significant ($p < 0.001$) decrease in (E_{max} 0.21 ± 0.004 g, n = 6) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax} normoxic group (Figures 6A, B, C).

CRC response curve induced by histamine in presence of EGCG (10 μM) was shifted to right with significant ($p < 0.001$) increase in EC_{50} (5.35 ± 0.04 μM) and with significant ($p < 0.001$) decrease in E_{Bmax} (0.49 ± 0.04 g, n = 6) in normoxic condition as compared to nontreated normoxic control (EC_{50} 5.01 ± 0.01 μM, E_{max} 1.22 ± 0.06 g; n = 6). Similarly, histamine induced CRC response curve elicited in presence of EGCG was shifted to right with significant ($p < 0.001$) decrease in (EC_{50} 4.99 ± 0.01 μM, E_{max} 0.20 ± 0.01g) in hypoxic rings in comparison with that of EC_{50} and E_{Bmax} of normoxic group (Figures 7A, B, C).

4. Discussion

The major observations are: (i) contractile response to 5-HT is greater than that of histamine, suggesting that this artery is more sensitive to serotonergic than histaminergic receptor. This further demonstrated that PA of *Ch* could be a good vascular model for evaluation of drugs acting on pulmonary artery, (ii) 5HT and histamine-induced vasotonic response were reduced by about 60% with experimental induction of acute hypoxia, (iii) a greater vasorelaxation effect was observed in 5HT-precontracted normoxic and hypoxic rings with naringenin, in histamine-precontracted in normoxic ring with EGCG and in histamine-precontracted hypoxic ring with quercetin indicating that all three polyphenols have potentials to control increased pulmonary vascular resistance in normoxic and hypoxic conditions.

In order to explain the effect of different polyphenols (quercetin, naringenin, EGCG) on vasotonic responses, 5HT concentration related contractile response was elicited either in absence or presence of quercetin or naringenin or EGCG in normoxic and hypoxic PA of *Ch*. In the present study, it was observed that quercetin reduced the 5HT induced maximal response by 44% without altering the affinity suggesting that quercetin caused inhibition of 5HT- induced vasotonic effect, either by modulating 5HT receptor mediated signalling mechanism as in cultured coronary arteries (Deng *et al.* 2014); in pulmonary artery smooth muscle cells (Liu *et al*., 2007) or activating vasorelaxation mechanism of endothelial /non-endothelial cells as in bovine aortic endothelial cell cultures (Mcduffie *et al*., 1999) or interfering the Ca^{+2} entry pathways in arterial tissues like in isolated cerebral and peripheral arteries from rats (Chang and Owman 1987) ; in dog saphenous vein (Sumner *et al*., 1992); in bovine pulmonary arteries (Alapati *et al*., 2007).

In presence of quercetin, 5HT-induced contractile response in hypoxic pulmonary arterial rings of *C. hircus* was further attenuated with decrease in affinity by 2.31 log unit and maximal response by 85%. Our observation, demonstrated that quercetin inhibited the 5HT receptor-mediated contractile response almost identical in normoxic and hypoxic pulmonary arterial rings. This may be due to significantly restoration or up-regulation of $5\text{-}HT_{2A}$ receptors and reduced the Akt and S6 phosphorylation by quercetin (Morales-Cano *et al.*, 2014). Quercetin could be useful in the treatment of hypoxia of PAH as treatment with quercetin alone substantially ameliorated hypoxia induced brain dysfunctions and acts like a neuroprotectant (Sarkar *et al*., 2012).

Naringenin caused a clear cut rightward shift of 5HT-contractile response curve with decrease in affinity by 0.32 and maximal contraction by 63% in normoxic pulmonary arterial rings. This observation demonstrated that naringenin inhibited the vasotonic response of 5HT with significant attenuation of affinity and maximal contractile responses. Such effect could be due to either interference of agonist-receptor interaction or inhibition signalling pathways of 5HT receptor activation in mediating contractile response or activating vasorelaxation pathways *via* modulation of $NO/PGI_2/ K_{ATP}$ channel/ Na^+ pump/ Ca^{+2} inactivation pathways as in the isolated mouse middle cerebral artery (Ni, 2004); in human, monkey and dog coronary artery (Toda, 1991). In hypoxic pulmonary arterial rings, naringenin inhibited the 5HT induced contraction with decrease in the affinity by about 1 log unit and maximal response by 93%. So, hypoxia further poteintiates the inhibitory effect of naringenin contractile response of 5HT in PA of *Ch*. This reduced vasoconstriction to 5HT observed in hypoxic PA of *Ch* may be attributed to the decreased HIF-1α and VEGF expression as in a murine model of hypobaric hypoxia (Sarkar *et al*., 2012) and may be mediated through serotongenic pathway (Dutt-Roy *et al*., 2015). The further inhibition of 5HT vasotonic response by naringenin could be mediated by modulation of signalling cascade or activation of vasorelaxation pathways which makes it suitable to be used as a drug in PAH combating hypoxia.

EGCG inhibited the 5HT induced contractile response with decease in affinity by 1.11 log unit and E_{Bmax} by 38% in normoxic and affinity by 0.66 log unit and maximum response by 69% in hypoxic PA of *Ch*. EGCG retains ability to reverse partially the affinity of 5HT receptor as modulated by hypoxia. It does not further poteintiate the inhibitory effects on 5HT contraction as in case of quercetin and naringenin, implying that EGCG possesses possible vasoprotective potential as EGCG preserves endothelial functions by reducing the endogenous nitric oxide synthase inhibitor level (Tang *et al*., 2006) and can extend help as a protective measure for PAH against hypoxia.

Concisely, the percentage of inhibition of serotonergic receptor-induced vasototonic response mediated by quercetin, naringenin and EGCG is greater in hypoxic than normoxic tissues. Our observations clearly demonstrated that polyphenols exhibited significant inhibitory effect on 5HT induced vasotonic response in pulmonary arterial rings of *C. hircus* in the increasing order of vasorelaxation potency, *i.e.*, naringenin > quercetin > EGCG in normoxic and hypoxic condition.

Histamine induced a concentration related contractile response in both normoxic and hypoxic pulmonary arterial rings of *C. hircus*. Concentration related curve of histamine was significantly shifted to right, decreased on affinity by 0.16 in hypoxic rings. As observed with 5HT, there was an identical inhibition of histamine contraction in pulmonary arterial rings of *C. hircus* under hypoxic condition. It could be predicted that hypoxia caused a downregulation and/or reduced function of histamine receptor that resulted in attenuation of function or expression of signalling molecules participated in histamine receptor coupling pathways as H_2 receptor is coupled to both adenylyl cyclase and phospholipase C through G_s and G_q proteins respectively (Delvalle and Gantz, 1997; Hill *et al*.,1997; Leurs *et al*., 1994) and then histamine initiates two pathways which result in either an increased intracellular cyclic AMP or Ca^{2+}

level (Fukushima *et al*., 1996; Kuhn *et al*.,1996; Wang *et al*., 1996) and histamine receptor activation leads to receptor desensitization (Smit *et al*., 1994).

Quercetin caused an inhibition of vasoconstriction of histamine reducing EC_{50} by -0.39 log unit and maximum relaxation by 36% in nomoxic rings. The maximal contractile response of histamine was augmented to 89% in hypoxic rings with 0.23 log unit reduction in affinity. This result demonstrated that quercetin exhibited a greater inhibitory effect in histamine mediated vasotonic response in hypoxic PA of *Ch* than normoxic ones as quercetin increases NO bioavailability in endothelial cells, plays a role in the vascular protective effects associated with improved endothelial cell function by inducing vasorelaxation through an eNOS phosphorylation process blocked by an increase in catalase activity, Ca^{2+} mediated eNOS dependent and independent pathways (Khoo *et al*., 2010) by which quercetin reduces inflammation and cerebral edema associated with altitude diseases without the side effects of steroid therapy (Patir *et al*. 2012) and is used as antioxidants used in the altitude sickness (Sarkar *et al*., 2012) in rats and thus paves way to be used against hypoxia in PAH.

Naringenin inhibited histamine induced contractile response with 51% and 83% attenuation of maximal contractile response with increase in affinity of histamine receptor in normoxic and hypoxic pulmonary arterial rings of *C. hircus*, respectively. This may be due to the mechanism involving the inhibition of formation and release of endogenous histamine in the gastric mucosa of rats is implicated in the protective effect of naringenin (Parmar, 1984). Also, grapefruit juice increases the bioavailability of H_1 antihistamines through their interaction in the intestines (Bartra, *et al.*, 2006) as inhibits CYP3A4 by the active metabolite naringenin (Criado *et al*., 2010) and strongly inhibits histamine release from rat mast cells to suppress allergic reaction through the inhibition of histamine release and is known as a histidine decarboxylase inhibitor (Yamamoto *et al*., 2014) which can also thus help to ameliorate PAH combating hypoxia.

ECCG also inhibited the histamine contraction with 60% and 84% attenuation of maximal contractile response with increase in affinity of histamine receptor in normoxic and hypoxic PA of *Ch*, respectively. This finding clearly demonstrates that vasocontration to histamine in PA of *Ch* is reduced in both normoxic and hypoxic conditions as the bioactive compound epigallocatechin-3-gallate (EGCG) targets histamine-producing cells producing great alterations in their behavior, proliferative potential, adhesion, migration, invasion potentials, as a potent inhibitor of the histamine-producing enzyme, histidine decarboxylase thus extends potent anti-inflammatory, antitumoral, and antiangiogenic effects (Melgarejo *et al.*, 2010) and can be helpful in improving blood circulation of hypoxic blood vessels in PAH.

The vasorelaxation of quercetin, naringenin and EGCG in histamine-precontracted rings is more in hypoxic than normoxic rings. Our observations clearly demonstrated that polyphenols exhibited significant inhibitory effects on histamine induced vasotonic response in pulmonary arterial rings of *C. hircus* in the order of potency, *i.e.*, EGCG> naringenin>quercetin in normoxic and quercetin >EGCG> naringenin in hypoxic condition.

5. Conclusion

PA of *Ch* can be used as a vascular prototype model to evaluate the mechanisms of vasoreactivity of different drugs and nutraceuticals in addition to the rodent model. While comparing the vasotonic responses of 5HT and histamine in PA of *Ch*, the PA of *Ch* shows more sensitivity to 5HT than histamine which indicates that there is a greater function and expression of 5HT receptor than histamine receptor. Hypoxia reduced the 5HT and histamine-induced contractile response by 60% that could be probably due to reduced function and expression or downregulation of these receptors. Polyphenols like quercetin, naringenin and EGCG exhibited vasorelaxation in both 5HT and histamine receptor activated contraction in both normoxic and hypoxic conditions. It is interesting to note that it could cause vasorelaxation where the vascular resistance is severely decreased. Hence, the use of these polyphenols could be effective in protecting the normal vasoreactivity during inflammatory damage of PA. In hypoxic PA where the vasotonic response is greatly diminished, these polyphenols could be used to improve blood circulation in the lungs.

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Conflict of interest

We declare that we have no conflict of interest.

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