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ORIGINAL ARTICLE

Curcumin Vasorelaxation in Uterine Artery of Goat (*Capra hircus*) is Mediated by Differential Activation of Nitric Oxide, Prostaglandin I_2, Soluble Guanylyl Cyclase, and Gap Junction Communication

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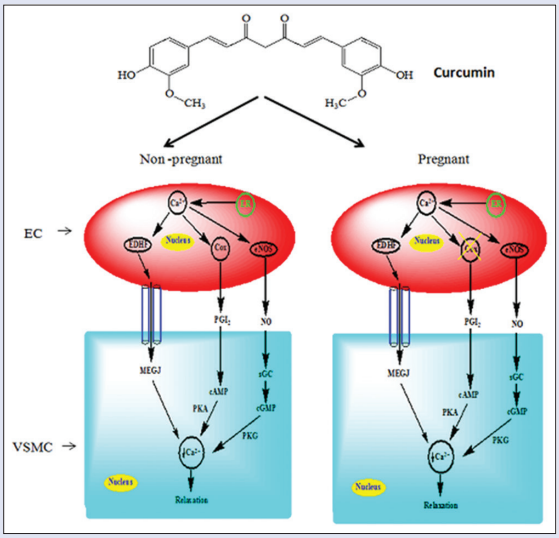
ABSTRACT

Background: Curcumin is a principal active constituent of *Curcuma longa* and has potential therapeutic application in various disease states. In this study, we investigated the mechanism of vasorelaxatory effects of curcumin in middle uterine artery (MUA) of both pregnant (P) and nonpregnant (NP) *Capra hircus* (*Ch*). **Materials and Methods:** The middle uterine arterial rings (MUA) were mounted in an automatic organ bath attached to a PowerLab data acquisition system. We analyzed the effect of curcumin on signaling mediators of endothelium-derived hyperpolarizing factor: nitric oxide (NO), soluble guanylyl cyclase (sGC), prostaglandin I_2 (PGI_2), and myoendothelial gap junctions (MEGJs). **Results:** The maximal vasorelaxation response to curcumin (1 ρM–100 μM) induced in phenylephrine- precontracted endothelium intact and denuded MUA rings were 42.58%, 25.12% in NP and 55.49%, 12.66%, in P Ch. In the presence of 1H-(1,2,4) oxadiazolo (4,3,-a) quinoxalin-1-one, the maximal curcumin-induced vasorelaxation (CVR) was inhibited to 20.65%, and 15.81% in MUA of NP and P *Ch*. In the presence of N.-nitro-L-arginine methyl, indomethacin and combination of both CVR were decreased to 20.49%, 12.47%, 12.82% in MUA ring of NP and to 40.60%, 52.55%, 46.53% in MUA ring of P *Ch*, respectively. The sensitivity of curcumin to MEGJ blockers carbenoxolone, 18β-glycyrrhetinic acid in causing vasorelaxation was 33.18%, 22.61% in MUA rings of NP, and to 15.76%, 18.54% in P *Ch*. **Conclusion:** In P *Ch*, endothelium-dependent vasorelaxation to curcumin is augmented by two-fold as that of MUA of NP. Endothelial vasorelaxation in MUA of NP is mediated through the activation of cyclooxygenase-PGI_2-cyclic adenosine monophosphate and endothelial NO synthase (eNOS)-sGC-cyclic guanosine monophosphate (cGMP) signaling with low activation of sGC and MEGJ. In contrast, vasorelaxation to curcumin is mediated through increased activity of sGC and MEGJ with major involvement of eNOS-NO-sGC-cGMP in MUA of P *Ch*. These studies have strong implications in the therapeutic targeting for hypertensive disorders.

Key words: Curcumin, hypertension, nitric oxide, uterine artery, vasorelaxation

SUMMARY

- Curcumin-induced vasorelaxation in middle uterine artery of both nonpregnant and pregnant *Capra hircus*. Curcumin-induced vasorelaxation in the middle uterine artery of nonpregnant *Capra hircus* is endothelium-dependent through cyclooxygenase-prostaglandin I_2 and endothelial nitric oxide synthase-nitric oxide pathways with an additional participation of myoendothelial gap junction. Endothelial nitric oxide synthase-nitric oxide pathway and myoendothelial gap junction play a vital role in middle uterine artery of pregnant *Capra hircus*. Data suggest a novel role of curcumin in the treatment of hypertensive disorders such as preeclampsia.



Abbreviations used: cAMP: Cyclic adenosine monophosphate; cGMP: Cyclic guanosine monophosphate; *Ch*: *Capra hircus*; COX: Cyclooxygenase; CVR: Curcumin-induced vasorelaxation; EC: Endothelial cell; ED−: Endothelium denuded; ED+: Endothelium intact; EDHF: Endothelium-derived hyperpolarizing factor; eNOS: Endothelial Nitric oxide synthase; IC_{50}: Half maximal inhibitory concentration; L-NAME: N_ω-Nitro-L-arginine methyl ester hydrochloride; MEGJ: Myoendothelial gap junctions; MUA: Middle uterine artery; NO: Nitric oxide; NP: Nonpregnant; ODQ: 1H-(1,2,4) oxadiazolo (4,3,-a) quinoxalin-1-one; P: Pregnant; PGI_2: Prostacyclin; PKA: Phosphokinase-A; PKG: Phosphokinase-G; pIC_{50}:-log (IC_{50}); R_{max}: Maximum relaxation; R_{Bmax}: Maximum relaxation in the presence of blocker; sGC: Soluble guanylyl cyclase; VSMC: Vascular smooth muscle cell.

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INTRODUCTION

Nitric oxide (NO) once described as the "Miracle Molecule" won the molecule of the year award in 1992. Robert F. Furchgott and colleagues were honored with a Nobel Prize for their invention of "NO as a signaling molecule in the cardiovascular system" in 1998.[1] NO plays a critical role in the maintenance of vascular tone through relaxing vascular smooth muscle cells. Three isoforms of NOS are now known to exist, two of which are constitutive and one of which is inducible by immunological stimuli,[2] namely endothelial NO synthase (eNOS) in vascular endothelium, nNOS in neurons, and iNOS in response to immunological stimuli.[3] The most well-distinguished vasorelaxation mechanism of NO is the stimulation of soluble guanylyl cyclase (sGC) in smooth muscle cells. Activated sGC catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP targets protein kinase G (PKG) in smooth muscle cells, activates the myosin light-chain phosphatase, which dephosphorylates smooth muscle myosin. This process results in loss of tonic contraction of the contractile apparatus which leads to vasorelaxation.[4]

Myoendothelial gap junctions (GJs) bridge endothelial cells (ECs) and smooth muscle cells. The hyperpolarization in smooth muscle cells is basically conducted from the ECs through myoendothelial GJs (MEGJs), since smooth muscle and ECs are coupled.[5] Connexins play specific roles in estradiol-17 β-treatment-regulated uterine function and placental development during early gestation.[6] In rat and guinea-pig small arteries, rabbit iliac arteries pharmacological blockade of GJs blunts endothelium-derived hyperpolarizing factor (EDHF)-mediated responses.[7] Under hypertensive conditions GJ-mediated response possibly dysfunctional as in spontaneously hypertensive rats (SHR), the protein expressions of connexins in ECs of the mesenteric arteries were altered compared to those in Wistar-Kyoto rats.[8]

During normal pregnancy, there is an increase in the activity of eNOS, cyclooxygenase (COX) and increased production of NO, prostaglandin I_2 (PGI_2), and EDHF. NO acts through the stimulation of the sGC that increases cGMP and PGI_2 increases cyclic adenosine monophosphate (cAMP) in smooth muscle respectively, which in turn decrease intracellular $Ca2^+$ and the myofilament sensitivity to $Ca2^+$. On the other hand EDHF activates K^+ channels in vascular smooth muscle cell membrane and causes hyperpolarization that results vasorelaxation. This mechanism resulted in smooth muscle relaxation and decreased peripheral resistance and arterial pressure. Hypertension is the most common health disorder of pregnancy and is reported to complicate up to 10% of pregnancies and is associated with increased maternal and neonatal morbidity and mortality.[9] In preeclampsia, there is increased release of placental cytokines such as tumor necrosis factor-alpha and interleukin-1 that inhibit the production of endothelium-derived relaxing factors and thus decrease smooth muscle relaxation.

Curcumin([1E,6E]-1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is an active principal constituent of turmeric, dried yellow powder obtained from the rhizomes of *Curcuma longa*. Curcumin has antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, antiproliferative, proapoptotic, and anti-atherosclerotic effects.[10] It has shown beneficial effects against cardiovascular diseases, neurodegenerative diseases, arthritis, allergy, inflammatory bowel disease, nephrotoxicity, AIDS, psoriasis, diabetes, multiple sclerosis, lung fibrosis, and as an alternative therapy for uterine leiomyoma.[11] Previous studies suggest that the pleiotropic effects of curcumin are reliant on its ability of interacting and regulation of multiple molecular targets.[12] These targets include inflammatory cytokines, transcription factors, growth factors, kinases, adhesion molecules, and apoptosis-related proteins.[13]

The role of EDH in vasorelaxation mechanism of curcumin is lacking in the uterine artery of any species. The study investigated the role of eNOS-NO-cGMP and COX-PGI_2 in relation to MEGJ in mediating endothelium-dependent hyperpolarization in middle uterine artery (MUA) of nonpregnant (NP) and pregnant (P) *Capra hircus* (*Ch*). Further, our study strongly supports that the administration of curcumin augments uterine blood flow (UBF) that can be helpful in maintenance of fetal health and diseases such as preeclampsia, a pregnancy complication characterized with high blood pressure.

MATERIALS AND METHODS

Ethical approval

This work was approved by Institutional Animal Ethical Committee (Registration No: 433/CPCSEA/CVS vide ID. No. 1586: (6)/CVS/dt. 03.05.2016 for conducting randomized *ex vivo* animal tissue experiments.

Preparation of middle uterine artery

Both NP and P uteri with broad ligament intact along with uterine artery were obtained in an aerated ice-cold (4°C–6°C) modified Krebs–Henseleit Saline solution to the laboratory. Secondary branch of uterine artery supplied to the uterine horn carefully cleared of fascia and connective tissue was cut into segments of circular rings measuring 1.5–2 mm in length. The arterial rings were then mounted between two fine stainless-steel L-shaped hooks and kept under a resting tension of 1.5 g in a thermostatically controlled (37.0°C ± 0.5°C) automatic organ bath (Pan Lab) of 20 mL capacity bubbled with carbogen (95% O_2 + 5% CO_2). Endothelium-denuded (ED−) rings were prepared by cotton swab method, i.e., endothelium removal was attained by gently passing a wet cotton thread 2–3 times in each direction through the lumen of the arterial ring.[14,15] The change in isometric tension was measured by a highly sensitive isometric force transducer (Model: MLT0201, AD Instruments Pty Ltd, Bella Vista, New South Wales, Australia) and analyzed using Lab Chart 7.1.3 software.

Curcumin-induced concentration-related response in phenylephrine-precontracted endothelium intact/endothelium-denuded middle uterine artery rings

Curcumin (1 ρM–100 μM) was added to bath cumulatively with an increment of 1.0 log unit at 4 min interval to relax the sustained contraction induced by phenylephrine (PE) (10 μM). Net tension (g) at each concentration of curcumin was recorded. The concentration-related response curves of curcumin were elicited and shift of the CRCs was compared between endothelium intact (ED+) and ED− groups. Maximum relaxation/maximum relaxation in presence of blocker (R_{max}/R_{Bmax}), mean threshold concentration, and pIC_{50}, were calculated for MUA rings for NP and *P* groups.

Curcumin-induced vasorelaxation in the presence of 1H-(1,2,4) oxadiazolo (4,3,-a) quinoxalin-1-one or N_ω-nitro-L-arginine methyl ester hydrochloride or indomethacin or N_ω-nitro-L-arginine methyl ester hydrochloride and indomethacin or carbenoxolone or 18β-glycyrrhetinic acid in phenylephrine-precontracted middle uterine artery rings

In order to examine curcumin (1 ρM–100 μM)-induced vasorelaxation involving the endothelium-dependent mechanisms, the arterial rings

were preincubated with 10 μM of 1H-(1,2,4) oxadiazolo (4,3,-a) quinoxalin-1-one (ODQ) or N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) or indomethacin or L-NAME and indomethacin for 15 min and carbenoxolone or 18β-glycyrrhetinic acid for 30 min prior to PE precontraction. Curcumin was added with increment of 1.0 log unit in a cumulative manner into the bath at 4 min interval after attaining a plateau contraction induced by PE. The concentration-related response curves of curcumin were elicited and shift of the CRCs were compared with nontreated control. R_{max}/R_{Bmax} (maximal relaxation), mean threshold concentration and pIC_{50} (-log of concentration causing 50% inhibitory response), were calculated for MUA rings of NP and P *Ch*.

Statistical analysis

The data were expressed as a percentage of relaxation at different concentration of curcumin in the absence or presence of pathways blockers and analyzed by the interactive nonlinear regression through the computer program Graph Pad Prism 5 (Graph Pad Prism Software, San Diego, CA, USA). Mean of R_{max}, R_{Bmax}, pIC_{50} was calculated for each set of concentration-related vasorelaxation curve and compared using unpaired Student's *t*-test with the help of Graph Pad Software Quick Calcs (San Diego, CA, U. S. A). $P < 0.05$ was considered statistically significant.

Drugs and chemicals

Curcumin (Sigma, USA), 18β-Glycyrrhetinic Acid (MP Biochemicals, India), NG–nitro-L-arginine methyl ester (L-NAME), ODQ (Cayman Chemical, USA), and carbenoxolone (Sigma, USA) were employed in this study. All the solutions were prepared fresh in triple-distilled water except for 18 βGA, ODQ which were dissolved in dimethyl sulfoxide, and indomethacin, dissolved in ethanol. Curcumin was prepared in 0.5N NaOH and phosphate-buffered saline.

RESULTS

Effect of curcumin (1 pM–100 μM) in phenylephrine (10 μM)-precontracted middle uterine artery rings

Table 1 represents the percentage maximal vasorelaxation (R_{max}) and pIC_{50} of curcumin in MUA of NP and P *Ch*. The representative raw traces show curcumin (1 pM–100 μM)-induced vasorelaxation elicited in PE-precontracted ED+ and ED– MUA rings of NP and P groups at Figure 1a and b, respectively. Curcumin (1 pM–100 μm) inhibited PE-induced sustained contraction in both NP and P *Ch* in a concentration-dependent manner. Curcumin-induced vasorelaxation (CVR) curve (R_{max} 42.58% ± 1.84%; pIC_{50} 8.83 ± 0.10) elicited in ED+ MUA rings of NP *Ch,* was shifted to right with significant ($P < 0.001$) decrease in R_{max} (25.12 ± 1.16%) and nonsignificant decrease in pIC_{50} (8.53 ± 0.14) in ED– rings of NP *Ch* [Figure 2a]. In MUA of P *Ch*, CVR curve (R_{max} 55.49% ± 1.69%; pIC_{50} 9.17 ± 0.10) elicited in ED+ rings was shifted to right with significant ($P < 0.001$) decrease in R_{max} (12.66% ± 1.66%) and pIC_{50} (5.99 ± 0.12) in ED– rings [Figure 2b].

Effect of N_ω-nitro-L-arginine methyl ester hydrochloride or indomethacin or N_ω-nitro-L-arginine methyl and indomethacin on curcumin-induced vasorelaxation

L-NAME, eNOS inhibitor and indomethacin, a COX inhibitor, and both attenuated curcumin vasorelaxation in PE precontracted MUA rings [Table 1]. In MUA of NP *Ch*, CVR curve (R_{max} 42.58% ± 1.84%; pIC_{50} 8.83 ± 0.10) was shifted right with significant ($P < 0.001$) decrease in R_{Bmax} (20.49% ± 1.02%) and

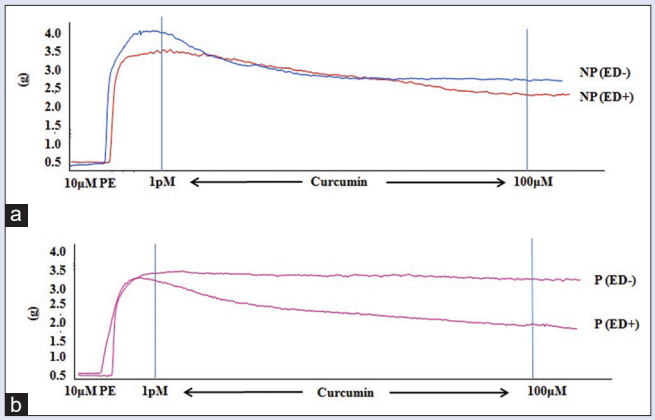

Figure 1: Representative raw trace showing effect of curcumin (1 ρM–100 μM) on Phenylephrine (10 μM)-induced sustained contraction in the presence of endothelium (Endothelium intact) and in the absence of endothelium (Endothelium denuded) in middle uterine artery ring of (a) nonpregnant *Capra hircus* and (b) pregnant *Capra hircus*

Table 1: Maximum relaxation and - log half-maximal inhibitory concentration of curcumin in endothelium intact or in endothelium-denuded or in absence (maximum relaxation) or in presence (maximum relaxation in presence of blocker) of N_ω-Nitro-L-arginine methyl ester hydrochloride or indomethacin or N_ω-Nitro-L-arginine methyl ester hydrochloride and indomethacin or 1H-[1,2,4] oxadiazolo[4,3,-a] quinoxalin-1-one or carbenoxolone or 18β-glycyrrhetinic acid in phenylephrine-precontracted middle uterine artery rings of nonpregnant and pregnant *Capra hircus*

Treatment	*n* value		R_{max}/R_{Bmax} (%)		pIC_{50}	
	NP	P	NP	P	NP	P
Control (ED+)	11	12	42.58±1.84	55.49±1.69	8.83±0.10	9.17±0.10
ED–	6	6	25.12±1.16[a]	12.66±1.66[a]	8.53±0.14	5.99±0.12[a]
L-NAME	6	6	20.49±1.02[a]	40.60±1.18[a]	9.05±0.17	11.39±0.31[a]
Indomethacin	6	6	12.47±1.07[a]	52.55±1.62	8.01±0.13[b]	10.13±0.15[a]
L-NAME + Indo	6	6	12.82±0.32[a]	46.53±1.75[b]	7.28±0.09[a]	8.89±0.14
ODQ	6	6	20.65±0.34[a]	15.81±0.99[a]	6.79±0.12[a]	5.97±0.09[a]
Carbenoxolone	6	6	33.18±0.96[a]	15.76±0.92[a]	6.94±0.13[a]	6.74±0.16[a]
18βGA	6	6	22.61±0.24[a]	18.54±0.5[a]	7.67±0.15[a]	8.98±6.74

[a]$P<0.001$; [b]$P<0.05$ represents the level of significance between the rows within each column. The values are expressed as mean±SEM. MUA: Middle uterine artery; *n* value: Total number of MUA rings used in the control or treatment groups; R_{max}: Maximum relaxation; R_{Bmax}: Maximum relaxation in presence of blocker; IC_{50}: Half maximal inhibitory concentration; pIC_{50} : –log (IC_{50}); SEM: Standard error of mean; L-NAME: N_ω-Nitro-L-arginine methyl ester hydrochloride; ODQ: 1H-[1,2,4] oxadiazolo[4,3,-a] quinoxalin-1-one; 18βGA: 18β-Glycyrrhetinic acid; NP: Nonpregnant; P: Pregnant; Indo: Indomethacin; ED+: Endothelium intact; ED–: Endothelium denuded

nonsignificant increase in pIC_{50} (9.05 ± 0.17) in presence of L-NAME, with significant ($P < 0.001$) decrease in R_{Bmax} (12.47% ± 1.07%) and significant ($P < 0.05$) decrease in pIC_{50} (8.01 ± 0.13) in the presence of indomethacin. In the presence of both L-NAME and indomethacin, the CVR curve was also shifted to right with significant ($P < 0.001$) decrease in R_{Bmax} (12.82% ± 0.32%) and pIC_{50} (7.28 ± 0.09) [Figure 3a]. In MUA of P *Ch*, CVR curve (R_{max} 55.49% ±1.69%; pIC_{50} 9.17 ± 0.10) was shifted to right with significant ($P < 0.001$) decrease in R_{Bmax} (40.60% ± 1.18%) and increase in pIC_{50} (11.39 ± 0.31) in the presence of L-NAME, with non-significant decrease in R_{Bmax} (52.55 ± 1.62%) and significant ($P < 0.001$) increase in pIC_{50} (10.13 ± 0.15) in the presence of indomethacin. In the presence of L-NAME and indomethacin, the CVR curve was shifted to right with significant ($P < 0.05$) decrease in R_{Bmax} (46.53% ± 1.75%) and nonsignificant decrease in pIC_{50} (8.89 ± 0.14) [Figure 3b].

Effect of 1H-(1,2,4) oxadiazolo (4,3,-a) quinoxalin-1-one on curcumin-induced vasorelaxation

In MUA of NP *Ch*, in the presence of ODQ (sGC inhibitor, 10 μM), CVR curve showed rightward shift with significant ($P < 0.001$) decrease

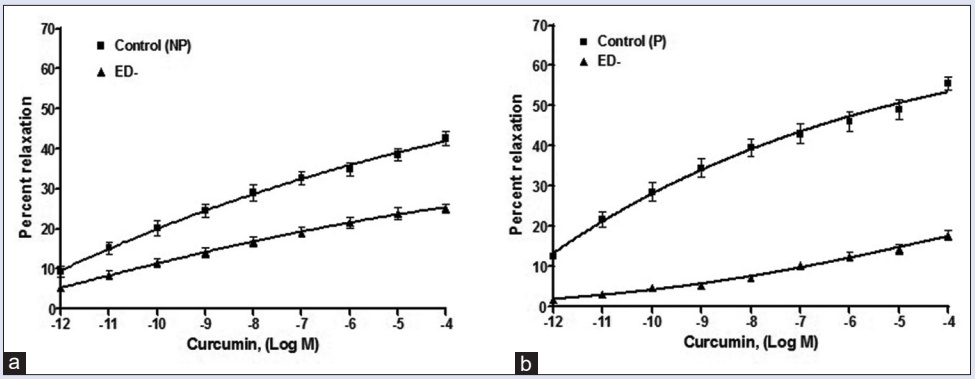

Figure 2: Curcumin (1 ρM–100 μM)-induced concentration-response curve elicited in the presence of endothelium (endothelium intact) and in the absence of endothelium (endothelium-denuded) in middle uterine artery ring of (a) nonpregnant *Capra hircus* and (b) pregnant *Capra hircus*

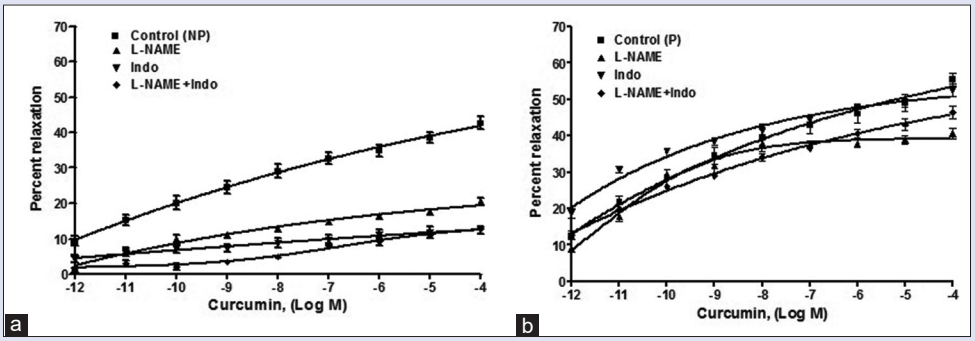

Figure 3: Curcumin (1 ρM–100 μM)-induced concentration-response curve elicited in absence (control) or in presence of N_{ω}-nitro-L-arginine methyl ester hydrochloride (10 μM) or indomethacin (Indo, 10 μM) or N_{ω}-Nitro-L-arginine methyl ester hydrochloride and indomethacin (N_{ω}-Nitro-L-arginine methyl + Indo, 10 μM) in middle uterine artery ring of (a) nonpregnant *Capra hircus* and (b) pregnant *Capra hircus*

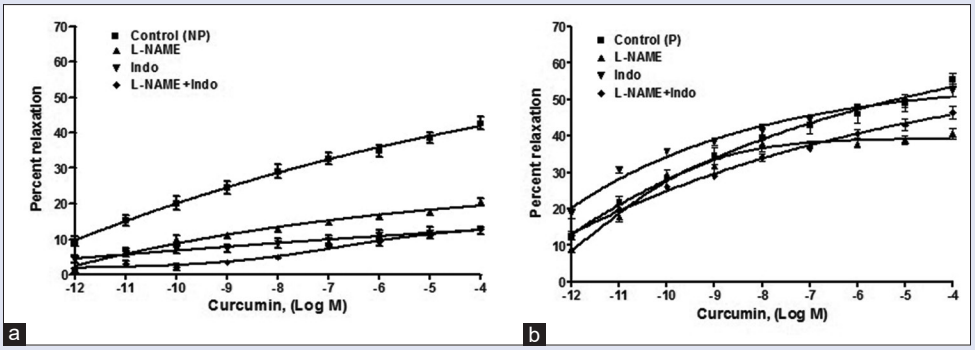

Figure 4: Curcumin (1 ρM–100 μM)-induced concentration-response curve elicited in absence (control) or in presence of 1H-(1,2,4) oxadiazolo (4,3,-a) quinoxalin-1-one (10 μM) in middle uterine artery ring of (a) nonpregnant *Capra hircus* and (b) pregnant *Capra hircus*

in R_{Bmax} (20.65% ± 0.34%) and pIC_{50} (6.79 ± 0.12) as compared to NP *Ch* control [Table 1 and Figure 4a]. In the presence of ODQ, CVR curve (R_{max} 55.49% ± 1.69%; pIC_{50} 9.17 ± 0.10) was shifted to right with significant ($P < 0.001$) decrease in R_{Bmax} (15.81% ± 0.99%) and pIC_{50} (5.97 ± 0.09) in MUA of P *Ch* [Table 1 and Figure 4b].

Effect of gap junction uncouplers carbenoxolone/18β-glycyrrhetinic acid

GJ uncouplers or connexin inhibitors such as carbenoxolone and 18β-glycyrrhetinic acid altered CVR significantly in MUA rings of both NP and P *Ch* [Table 1]. In the presence of carbenoxolone and 18β-glycyrrhetinic acid, the CVR curve was inhibited with significant ($P < 0.001$) decrease in R_{Bmax} and pIC_{50} (33.18% ± 0.96% and 6.74 ± 0.16) and (22.61% ± 0.24% and 7.67 ± 0.15), respectively, in MUA of NP *Ch* [Figure 5a]. In MUA of P *Ch,* carbenoxolone inhibited the vasorelaxation to curcumin with rightward shift of CVR curve with significant ($P < 0.001$) decrease in R_{Bmax} and pIC_{50} (15.76% ± 0.92%, 6.94 ± 0.13). 18β-glycyrrhetinic acid caused CVR curve significant ($P < 0.001$) decrease in R_{Bmax} (18.54% ± 0.50%) and nonsignificant increase in pIC_{50} (8.98 ± 6.74) [Figure 5b].

DISCUSSION

It has been well accepted that the mechanisms of vasorelaxation to curcumin varies with the location of blood vessels and species of animals.[16-20] Due to lack of literature it is not known whether curcumin has potential to cause vasorelaxation in uterine artery, if it causes what are the underlying possible signalling mechanisms involved in mediating vasorelaxation. To answer these questions, the vasorelaxation effect of curcumin was elicited in MUA of both NP and P goat in the presence of endothelial vasorelaxation signaling pathways inhibitors. The most important findings are (i) The maximal vasorelaxation obtained from CVR curve elicited in PE-precontracted ED + MUA rings was greater in P *Ch* than that of NP *Ch* [Table 1] suggesting a greater vasorelaxation action of curcumin in MUA of P *Ch*. (ii) Endothelium removal attenuated the maximal CVR in MUA of P more than NP *Ch*. This finding clearly demonstrate that endothelium-dependent vasorelaxation to curcumin is greatly augmented in MUA of P than NP *Ch*. (iii) L-NAME, indomethacin, L-NAME + Indomethacin decreased the maximal CVR in MUA ring of NP as compared to MUA ring of P *Ch* [Table 1]. These observations suggest that curcumin activates COX predominantly and eNOS moderately in NP *Ch*. In contrast, curcumin activates only eNOS moderately but not COX raising the possibility of involvement of other endothelium-dependent mechanisms in MUA of P *Ch*. (iv) In the presence of ODQ, the maximal CVR was inhibited in MUA of NP and P *Ch* indicating that increased blocking effect of sGC by curcumin in MUA of P *Ch*, (v) carbenoxolone, 18β-glycyrrhetinic acid reduced the maximal CVR in MUA rings of NP and in P *Ch* demonstrating an increased blocking effect of MEGJ uncouplers in MUA of P *Ch*.

Vasodilation effect of curcumin has been reported in rat aorta,[16] porcine coronary arteries,[17] rabbit basilar arteries,[18] goat ruminal artery,[19] and rat mesenteric arteries.[20] Hexahydrocurcumin (HHC) relaxed the ED+ rat aortic rings precontracted with PE and KCl in a concentration-dependent manner and no effect observed in the absence of endothelium suggesting that vasorelaxation to HHC is endothelium-dependent.[21] The administration of dietary curcumin for 1 month remarkably restored the impaired cerebrovascular endothelium-dependent vasorelaxation in aging SD rats.[22] In the same study, it was shown that curcumin promoted eNOS and AMPK phosphorylation, UCP_2 upregulation and reduced ROS production in the cultured ECs prepared from the cerebral arteries of aging SD rats further demonstrated that curcumin interacts with several targets of vasorelaxation pathways.

Curcumin-induced maximal vasorelaxation in ED+ MUA rings of NP and P *Ch* was attenuated in that of ED− rings. Hence, the maximal percentage vasorelaxation to curcumin is endothelium-dependent in uterine artery of NP and P *Ch*. To rule out the differential activation of NO, PGI_2, or EDHF by curcumin in MUA rings of NP or P *Ch*, CVR curve was elicited in the absence or presence of eNOS, COX inhibitors. L-NAME, indomethacin, and L-NAME+Indomethacin, significantly decreased the maximal CVR in MUA ring of NP *Ch*. The percent abolition of CVR following blockade of eNOS is almost equal to the CVR abolished due to removal of endothelium. In contrast, the percentage abolition of CVR following blockade of COX or both eNOS and COX is greater than the CVR due to endothelium. These findings clearly demonstrated that curcumin vasorelaxation is mediated in part through the activation of eNOS-NO signaling. Curcumin activates eNOS that increases the turnover of NO in the endothelium of MUA which is diffusing to the vascular smooth muscle cells where it is interacting with sGC to activate cGMP and cause vasorelaxation. In order to examine the involvement of sGC, CVR curve was elicited in the presence of sGC blocker ODQ. ODQ almost inhibited the maximal CVR, and this is almost identical to either the percentage vasorelaxation abolished with removal of endothelium or blockade of eNOS. Hence, one of the endothelial mechanisms of vasorelaxation to curcumin is mediated through the activation of eNOS-NO-sGC-cGMP signaling pathways.[17]

In MUA of P *Ch,* L-NAME, indomethacin both L-NAME and indomethacin inhibited the maximal CVR in MUA ring of P *Ch* indicating that CVR is mediated by activation eNOS in part but not COX. While comparing the attenuated percentage of vasorelaxation to curcumin in endothelium-denudated rings (42%) with that of

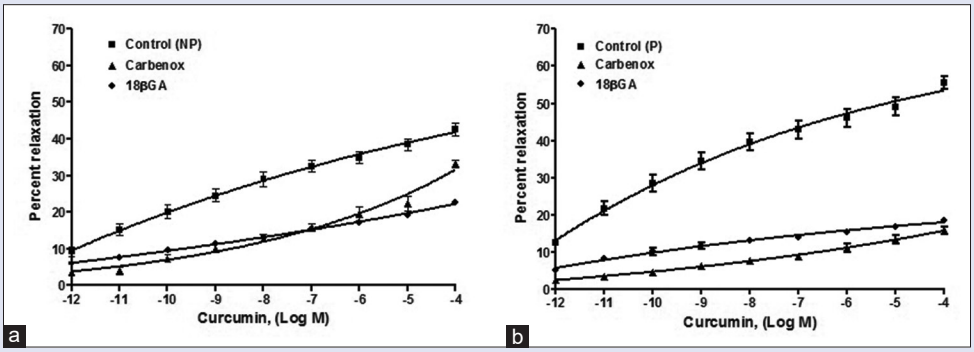

Figure 5: Curcumin (1 ρM–100 μM)-induced concentration-response curve elicited in the absence (control) or presence of carbenoxolone (Carbenox, 10 μM) or 18β-Glycyrrhetinic acid (18β-GA, 10 μM), in middle uterine artery ring of (a) nonpregnant *Capra hircus* and (b) pregnant *Capra hircus*

abolished CVR in the presence of L-NAME or/and indomethacin, it can be stated that a greater proportion of curcumin vasorelaxation is mediated by the activation of endothelium-dependent eNOS–NO mechanisms. The endothelium-dependent mechanism has been reported in coronary artery of dogs and humans[23] and rat aorta.[24] L-NAME insensitive mechanism is observed in cerebral vasculature of mice[25] and the endothelium-dependent, eNOS-independent inhibition is caused by NO produced by cytochrome P450 reductase in the endothelium of the SHR aorta.[26] In addition, eNOS, EDHF, and COX contribute to endothelium-dependent relaxation in the rat hepatic artery.[27] In $PGF_{2\alpha}$-precontracted porcine coronary arterial rings, curcumin (10^{-11}–10^{-5} mol/l) vasorelaxation significantly reduced by removal of endothelium, and by the addition of L-NMMA, but not by indomethacin suggesting that curcumin might activate NO and cGMP but not COX-PGI_2.[17] Our observation showed that about 43% of vasorelaxation to curcumin is endothelium-dependent. In contrast, only 15% of the endothelium-dependent vasorelaxation is mediated by eNOS-NO pathways. Hence, it is quite possible that a major component endothelial vasorelaxation to curcumin could be mediated by mechanism not involving eNOS and COX. Considering that the direct amplification of sGC activity in vascular smooth muscle cell (VSMC) by curcumin could be another additional mechanism to cause vasorelaxation through cGMP pathways as the CVR was attenuated in the presence of ODQ, a selective blocker of sGC. We observed that the maximal CVR was attenuated to 16% by ODQ indicating that the sensitivity of sGC to ODQ is greatly increased. The possible explanation for the increased sensitivity of sGC in the endothelium-dependent vasorelaxation to curcumin could be arising due to increase in the affinity or expression of sGC for binding of NO which in turn augmented the turnover of cGMP in the VSMC of MUA of P *Ch*. In goat ruminal artery, ODQ (10 μM) potently blocked curcumin (10 nM–100 μM)-induced vasorelaxation in dose-dependent manner in 5-HT and NA-induced contraction suggesting curcumin at least in part, act through direct activation of sGC-mediated cGMP pathway followed by opening of K^+ ion channel.[19] In accordance to the findings made in goat ruminal artery, it could be convincing to explain that curcumin probably increases the sensitivity of sGC for NO binding thereby augmenting turnover of cGMP in MUA of P *Ch*. In conclusion, the endothelium-dependent and eNOS independent vasorelaxation to curcumin observed in isolated MUA of both NP and P *Ch* is mediated almost exclusively by the activation of sGC-cGMP pathways.

In vascular relaxation, PGI_2 and its analogs have been associated with concomitant hyperpolarization of the smooth muscle cells via opening of K^+-channels. Basing on the type, location, and species the opening of various populations of K^+-channels are involved.[28] In numerous vascular bed, PGI_2 has been considered as an endothelium-derived hyperpolarizing substance.[29] The blockade of COX reduced the endothelial production of PGI_2 leading to attenuation of vasorelaxation to curcumin in MUA. The blockade of COX or eNOS and COX reduced the CVR by 30% and by <5% in MUA of NP and P *Ch* indicating that COX-PGI_2 signaling is predominantly and minimally activated by curcumin in MUA of NP and P *Ch*, respectively. In MUA of NP *Ch*, the percentage of vasorelaxation abolished by COX blockade exceeds the percentage of vasorelaxation due to removal of endothelium. Hence, it is convincing to explain that curcumin activates PGI_2 in the endothelium that is associated with the hyperpolarization in the smooth muscle cells via opening of K^+-channels as reported in several other vascular bed.[28,29] In goat MUA, curcumin showed differential sensitivity to different K^+ channels, caused direct hyperpolarization and vasorelaxation of VSMCs by opening of K_{Ca}, K_{ATP}, K_{ir}, K_V channels in NP *Ch* and K_{Ca}, and K_{ir} channels in P *Ch*.[30] In rat superior mesenteric artery, it has been reported that curcumin-mediated vasorelaxation involves indomethacin-sensitive COX-PGI_2 but not L-NAME and ODQ sensitive pathways. Similarly, curcumin caused vasorelaxation in superior mesenteric arterial rings through an endothelium-dependent pathway involving prostanoids and also through an endothelium independent pathway, opening of K^+-channels, blockade of Ca^{2+}-influx, and intracellular Ca^{2+}.[20] In MUA of NP goat, one of the predominant endothelium-dependent mechanism for CVR involves prostanoids (COX-PGI_2-cAMP signaling pathways) that could be associated with hyperpolarization in smooth muscle cells through opening of opening of K^+-channels, blockade of Ca^{2+}-influx, and intracellular Ca^{2+}.

GJs are compactly packed clusters of intercellular channels between endothelial- ECs and endothelial-VSM cells that connects cells electrically and metabolically.[31] The GJ directly couples the cytoplasm of adjacent cells to facilitate passage of ions, signaling molecules, and second messengers.[32] During pregnancy, the EC adaptation engages in significant role in the modulation of vascular resistance and UBF through increase in the PGI_2 and NO.[33] MEGJ plays a critical role in the elevations of these endothelial vasodilators through cell-cell signaling process by enhancement of cell connectivity.[34] The functional role of MEGJ in CVR in MUA of NP and P *Ch* is not elucidated. In consideration of potential endothelium-dependent vasorelaxation to curcumin in MUA of NP *Ch* and its further augmentation in that of P *Ch*, it is expected that MEGJ could be playing role in vasorelaxation mechanism of curcumin. In order to examine this, CVR curve was elicited in presence of two GJ uncouplers namely, carbenoxolone, and 18β-glycyrrhetinic acid. Both the GJ uncouplers reduced the maximal CVR in MUA rings of NP and P *Ch*. This clearly showed that in MUA the sensitivity to these GJ uncouplers is increased from NP to P *Ch*. Further, the attenuation of percentage maximal CVR in absence of endothelium is almost close to the inhibition by these MEGJ uncouplers indicating that endothelium-dependent vasorelaxation to curcumin could be involving the MEGJ. Reports showed that curcumin-induced increase efflux of GSH in astrocyte cells of SD rat attenuated by GJ uncoupler carbenoxolone.[35] Dietary feeding with curcumin and tetrahydrocurcumin reduced the level of connexin-43 (molecule of GJs), in cancer cells of experimental colon carcinoma in mice.[36] These findings clearly suggest that the MEGJ plays functional role in the transportation of small signaling molecules and ions in subserving curcumin-mediated vasorelaxation in ED+ MUA of NP and this is greatly increased in P *Ch*. It is most likely that curcumin might be activating MEGJs in ED+ uterine vascular tissues that increase entry of Ca^{2+} from VSMC to endothelium to activate eNOS and COX as reported by Stridh *et al.*,[35] in part or facilitating the transportation of PGI_2 to VSMC to cause hyperpolarization. In several vascular bed-like goat superior mesenteric artery.[37] GJ has been reported as one of the EDHFs. The activation of GJ communication in MUA observed in our present findings clearly indicates that EDHFs could be one of the targets for vasorelaxation to curcumin.

CONCLUSION

These studies have provided ample evidence that curcumin caused a differential vasorelaxation in MUA of NP and P *Ch*. Endothelium-dependent vasorelaxation mechanisms of curcumin are greatly increased from nonpregnancy to pregnancy which may be arising from vascular remodeling leading to endothelial adaptation. In MUA of NP and P *Ch*, increase in the sensitivity of eNOS in the ECs, sGC in the VSMC and GJ communications by curcumin clearly depicts the involvement of eNO-sGC-cGMP signaling mechanisms in vasorelaxation. Apart from these mechanisms, PGI_2-cAMP signaling pathway is potential mechanism in the vasorelaxation to curcumin in NP *Ch*. These studies indicate strong prospects of

curcumin in the therapeutic management for hypertensive disorders in pregnancy.

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Conflicts of interest

There are no conflicts of interest.

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