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Original article

Curcumin and nanocurcumin differentially activate K^+ channels in uterine artery of non-pregnant and pregnant *Capra hircus*

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Abstract

K^+ channels activation in vascular bed cause vasorelaxation and decrease in hypertension. In the present study, we compared different K^+ channels (K_{Ca}, K_{ATP}, K_{ir}, K_V) activation by curcumin and nanocurcumin, in the vasorelaxation mechanism of uterine artery, obtained from non-pregnant (NP) and pregnant (P) C*apra hircus* (*Ch*). Middle uterine artery (MUA) rings were mounted in an automatic organ bath containing 20 ml MKHS, maintained at pH 7.4. Phenylephrine (PE, 10 µM) was added to bath to induce a sustained contraction. Curcumin and nanocurcumin (1pM-100 µM) were added to bath in a cumulative concentration manner to inhibit PE induced sustained contraction either in absence or presence of different K^+ channel blockers like glibenclamide, tetraethylammonium (TEA), 4-aminopyridine (4-AP) and barium. Contractile response was recorded isometrically by highly sensitive force transducer automatic organ bath connected to powerlab and analysed using Labchart 7.1.3 software. The maximal vasorelaxation in PE-precontracted ED + MUA rings with curcumin/nanocurcumin (1pM-100 µM) was NP 42.58%, 40.36% and P *Ch* 55.49%, 44.09%. In presence of K^+ channel blockers glibenclamide, TEA, 4-AP and barium, CVR was inhibited to 20.31%, 17.68%, 34.13%, 31.94% in MUA of NP and to 53.10%, 16.67%, 58.87%, 48.64% in MUA of P *Ch*, respectively. Similarly, NCVR was decreased to 32.01%, 25.82%, 21.75%, 27.60% in MUA of NP and to 56.42%, 30.70%, 34.38%, 36.96% in MUA of P Ch, respectively. The sensitivity of curcumin and nanocurcumin to different K^+ channels in causing vasorelaxation is in the decreasing order of $K_{Ca} > K_{ATP} > K_{ir} > K_V$ and $K_V > K_{Ca} > K_{ir}$ in NP and $K_{Ca} > K_{ir}$ and $K_{Ca} > K_V$ in P Ch, respectively. Our result suggests that (i) both Curcumin and nanocurcumin predominantly and precisely activate K_{Ca} in pregnant uterine artery and differentially activate K_{ATP},K_{ir},K_V in non-pregnant uterine artery, (ii) one of the mechanism of vasorelaxation due to curcumin and nanocurcumin is attributed to differential opening of different K^+-channels. Administration of curcumin and nanocurcumin in therapeutic dose could control hypertension in pregnancy (pre-eclampsia).

Key words: Uterine artery, potassium channels, pregnancy, curcumin, nanocurcumin

1. Introduction

The K^+ channels are present in plasma membrane of arterial smooth muscle cells, plays crucial role in the regulation of vascular tone (Nelson and Quayle, 1995; Jackson, 2005). The outflow of K^+ through these channels, hyperpolarizes the membrane and, thereby inhibits the entry of Ca^{2+} causing relaxation of blood vessels (Ko *et al*., 2008). Depolarization is caused by the inhibition of K^+ channels and activation of Ca^{2+} channels, which in turn causes vasoconstriction. Pregnancy is associated with an increase in uterine blood flow and a decrease in uterine vascular resistance. The uterine vasculature undergoes significant remodelling to accommodate the dramatic increase in uteroplacental blood flow that is requisite for normal pregnancy outcome (Sladek *et al*., 1997). Evidence suggests K^+ channels may play a key role in the adaptations of uterine circulation to pregnancy (Rosenfeld *et al*., 2009; Xiao *et al*., 2010). Modulation of K^+ channels activity in uterine vasculature may be considered as one of the important mechanisms underlying chronic hypoxia-mediated uterine vascular dysfunction during pregnancy (Zhu *et al*., 2013).

Four different types of potassium channels distinguished in arterial smooth muscle. Voltage-dependent channels (K_V), Ca^{2+}-activated channels (large-conductance, BK_{Ca}; intermediate-conductance, IK_{Ca}; and small conductance, SK_{Ca}), ATP-dependent channels (K_{ATP}) and inwardly rectifying channels (K_{ir}) (Jackson, 2005; Hill *et al*., 2010; Hayabuchi *et al*., 2011). The involvement of different types of K^+ channels has been evaluated by the use of channel-specific blockers. The most commonly used blockers of K^+ channels are tetraethylammonium (TEA) and $BaCl_2$ as nonselective inhibitors; glibenclamide, an inhibitor of K_{ATP} channels; aminopyridine (4-AP), which blocks K_V channels; and iberiotoxin and charybdotoxin, which block BK_{Ca} channels. In addition, TEA, $BaCl_2$, and apamin have been used to block BK_{Ca}, K_{ir}, and SK_{Ca} channels, respectively. Endothelium-dependent vasodilators, nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF), stimulate K^+ channels opens the K^+ efflux and causes hyperpolari-zation, which decreases the opening of voltage-gated Ca^{2+} channels to reduce Ca^{2+} influx, resulting in vascular relaxation (Baranowska *et al*., 2007).

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To investigate EDHF-mediated responses pharmacological inhibition of K^+ channels has been useful since hyperpolarization is caused by the opening of K^+ channels (Kang, 2014).

Curcumin, a hydrophobic polyphenol, is a principal active constituent of turmeric obtained from the rhizomes of *Curcuma longa* (Stanic, 2017). Evidence suggests that the pleiotropic effects of curcumin are dependent on its capacity of interacting and regulation of multiple molecular targets. Thus, due to its efficacy and regulation of multiple targets, as well as its safety for human use, curcumin has received considerable interest as a potential therapeutic agent for the prevention and/or treatment of various malignant diseases, arthritis, allergies, Alzheimer's disease and an alternative therapy for uterine leiomyoma (Tsuiji *et al*., 2011; Yallapu *et al*., 2015). Nanoformulations of curcumin exhibited advanced therapeutic benefits over the free curcumin in overcoming the problems such as poor bioavailability and prolonged QT interval (Ranjan *et al*., 2013). K^+ channels activity in the presence of curcumin was reported in rat superior mesenteric artery (Zhang *et al*., 2017), guinea pig gallbladder strips (Kline and Karpinski, 2015), goat ruminal artery (Dash and Parija, 2013), rabbit coronary smooth muscle cells (Hong *et al*., 2013), indomethacin-induced gastric injury rat model (Díaz-Triste *et al*., 2014) and bovine adrenal zona fasciculate (AZF) cells (Liu *et al*., 2006).

Information on the relative effect of curcumin and nanocurcumin on activation of different K^+ channels is almost lacking in any uterine artery. Therefore, the aim of this study was to evaluate the effect of different K^+ channels on curcumin and nanocurcumin in middle uterine artery of NP and P *Ch*. Our findings would provide evidences for the first time on the activation of K^+ channels by curcumin and nanocurcumin in inducing vasorelaxation in MUA of NP and P *Ch*. Further, present study will predict the potential of these nutraceutics in controlling hypertension in pregnancy (pre-eclampsia).

2. Materials and Methods

2.1 Ethical guidelines

This work has been 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.

2.2 Preparation of middle uterine artery and functional study

Non-pregnant and pregnant uterus with broad ligament intact along with uterine artery were obtained in an aerated ice-cold (4-6°C) Modified krebs-henseleit saline (MKHS) solution (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 11.9, KH_2PO_4 1.2 and dextrose 11.1, (*p*H 7.4). Secondary branch of uterine artery supplied to the uterine horn carefully cleared of fascia and connective tissue in MKHS solution under continuous aeration. The arteries were cut into segments of circular rings measuring 1.5-2 mm in length were then mounted between two fine stainless steel L-shaped hooks and kept under a resting tension of 1.5 gm in a thermostatically controlled (37.0 ± 0.5 ºC) automatic organ bath (Pan Lab) of 20 ml capacity bubbled with carbogen (95% O_2 + 5% CO_2). The change of isometric tension was measured by a highly sensitive isometric force transducer (Model: MLT0201, AD instrument, Australia) and analysed using chart 7.1.3 software.

2.3 PE-induced contraction and its inhibition by curcumin/nanocurcumin in absence or presence of different K^+ channels blockers in MUA rings of NP and P *Ch*

In order to examine differential sensitivity of K^+ channels in curcumin/nanocurcumin (1 pM-100 μM)-induced vasorelaxation response (CVR/NCVR), the arterial rings were pre incubated with glibenclamide (1 μM), TEA (10 mM), 4-AP (10 μM) or barium (10 μM) for a period of 30 min prior to PE precontraction curcumin/nanocurcumin 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/nanocurcumin were elicited and shift of the CRCs were compared with non treated control. R_{max}/ R_{Bmax}, mean threshold concentration and pIC_{50}, were calculated for MUA rings of NP and P *Ch*.

2.4 Data analysis

The data were expressed as percentage of the maximum relaxation to against obtained in the absence of antagonist (control) and analyzed by the interactive non-linear regression through the computer program Graph Pad Prism (Graph Pad Prism Software, San Diego, CA, USA). R_{max} / R_{Bmax}, mean threshold concentration and $-\log IC_{50}/pIC_{50}$ were calculated through Graph Pad Prism. GraphPad Quick Calcs '*t*' test was used to calculate the *p* value to determine the level of significance and to analyse the data. A '*p*' value < 0.05 and <0.001 were considered statistically significant.

2.5 Drugs

Tetraethylammonium (Sigma Aldrich, India), curcumin and nanocurcumin (Gifted by Dr. B.P. Mohanty, CIFRI, ICAR, India), glibenclamide (Sigma, USA), 4-Aminopyridine (HiMedia, India), barium chloride (Qualigens, India) were employed in this study.

3. Results

3.1 Effect of K^+ channel blockers on CVR

Table 1 presents the percent mean maximal vasorelaxation (R_{max}) and pIC_{50} of curcumin in absence or presence of K^+ channel blockers in MUA of NP and P *Ch*. Figures (1A, 1C and 1E) and Figures (1B, 1D and 1F) shows representative trace for effect of curcumin (1pM-100 μM) on PE (10 μM)-induced contraction in absence (control) or in presence of glibenclamide (glyben,1 μM) or TEA (10 mM) in MUA ring of NP and P *Ch* groups, respectively.

Table 1: R_{max} and pIC_{50} of curcumin (1pM-100 μM)-induced vasorelaxation on PE (10 μM) precontracted MUA rings in absence (control) or presence of glibenclamide (1 μM) or tetraethylammonium (TEA, 10 mM) or 4-aminopyridine, (4-AP, 10 μM) or barium (10 μM) in MUA of NP or P *Ch*. The values are expressed as Mean ± SEM, N = total numbr of MUA rings used in the experiments

Treatment	N Value		R_{max}/RB_{max} (%)		pIC_{50}	
	NP	P	NP	P	NP	P
Control (Curcumin)	11	12	42.58 ± 1.84	55.4 ± 1.69	8.83 ± 0.10	9.17 ± 0.10
Glibenclamide	6	6	20.31 ± 0.66[a]	53.10 ± 2.88	8.27 ± 0.16[b]	7.96 ± 0.17[a]
TEA	6	6	17.68 ± 0.83[a]	16.67 ± 0.29[a]	6.78 ± 0.12[a]	6.57 ± 0.11[a]
4-AP	6	6	34.13 ± 0.65[b]	58.87 ± 0.48	7.06 ± 0.09[a]	9.14 ± 0.15
Barium	6	6	31.94 ± 1.07[b]	48.64 ± 0.58[b]	8.23 ± 0.15[b]	8.69 ± 0.16[b]

[a]($p<0.001$), [b]($p<0.05$) represents level of significance between the rows within each column (with respective controls)

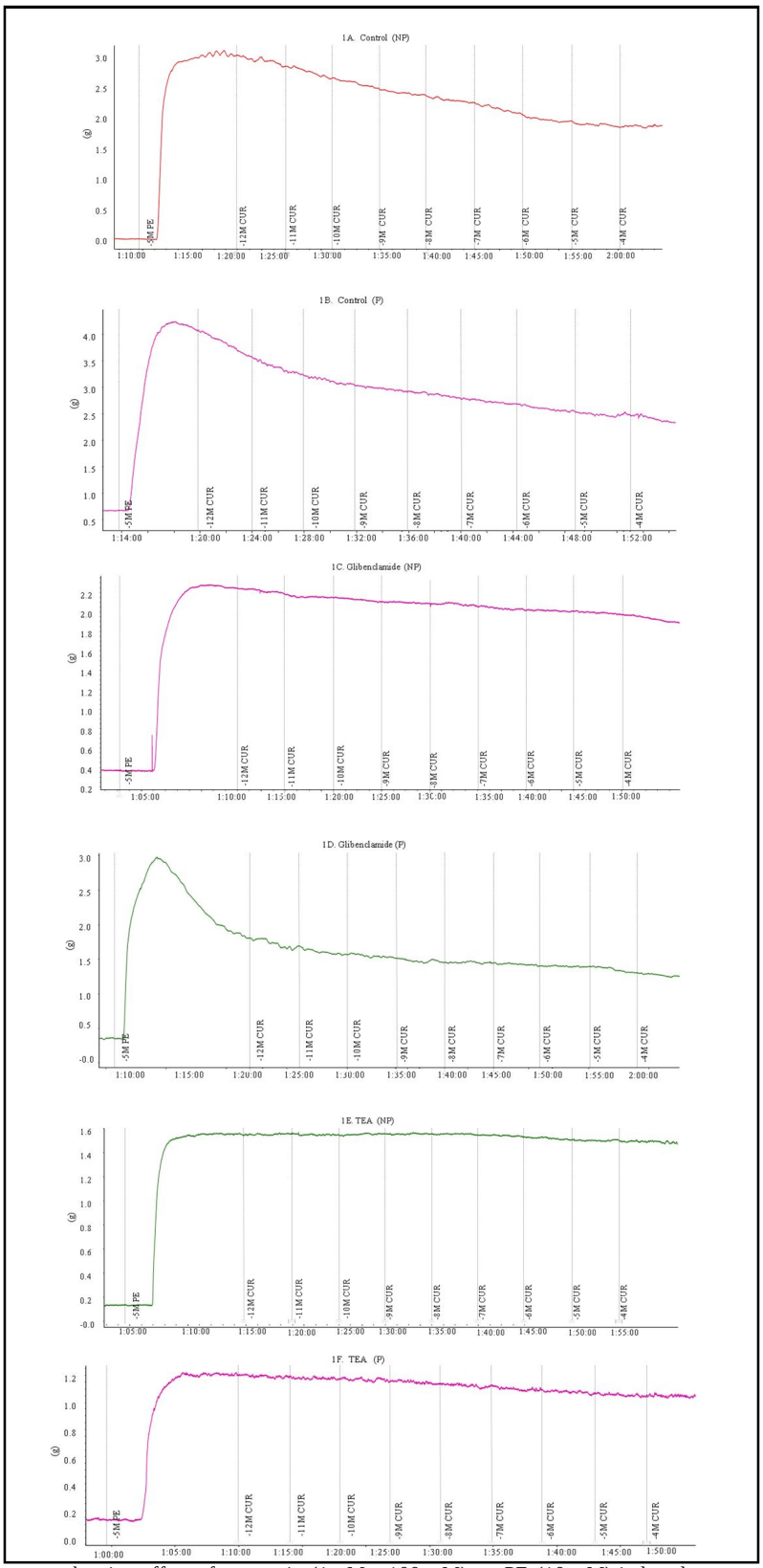

Figure 1: Representative raw trace showing effect of curcumin (1 pM - 100 µM) on PE (10 µM)-induced contraction in absence (Control) or presence of glibenclamide (1 µM), TEA (10 mM) in MUA ring of NP and P *Ch*. Vasorelaxation effect of curcumin (control) and its ameliorative effect by glibenclamide, TEA is shown at Figures 1A, 1C, 1E in NP and Figures 1B,1D,1F in P groups.

CVR curve (R_{max}: 42.58 ± 1.84, pIC_{50}: 8.83 ± 0.10) was shifted to right with significant (p<0.001) decrease in R_{Bmax} (20.31 ± 0.66 %) and significant (p<0.05) decrease in pIC_{50} (8.27 ± 0.16) in presence of glibenclamide (1 μM), significant (p<0.001) decrease in R_{Bmax} (17.68 ± 0.83%) and pIC_{50} (6.48 ± 0.12) in presence of TEA (10 mM), significant (p<0.05) decrease in R_{Bmax} (34.13 ± 0.65%) and significant (p<0.001) decrease in pIC_{50} (7.06 ± 0.09) in presence of 4-AP (10 μM), significant (p<0.05) decrease in R_{Bmax} (31.94 ± 1.07 %) and pIC_{50} (8.23 ± 0.15) in presence of barium (10 μM) (Figure 2A). CVR curve (Rmax: 55.49 ± 1.69, pIC_{50}: 9.17 ± 0.10) showed non-significant decrease in R_{Bmax} (53.10 ± 2.88%) and significant (p<0.001) decrease in pIC_{50} (7.96 ± 0.17) in presence of glibenclamide, significant (p<0.001) decrease in R_{Bmax} (16.67 ± 0.29%) and pIC_{50} (6.57 ± 0.11) in presence of TEA, non- significant increase in R_{Bmax} (58.87 ± 0.48%) and decrease in pIC_{50} (9.14 ± 0.15) in presence of 4-AP, significant (p<0.05) decrease in R_{Bmax} (48.64 ± 0.58%) and pIC_{50} (8.69 ± 0.16) in presence of Barium. (Figure 2B).

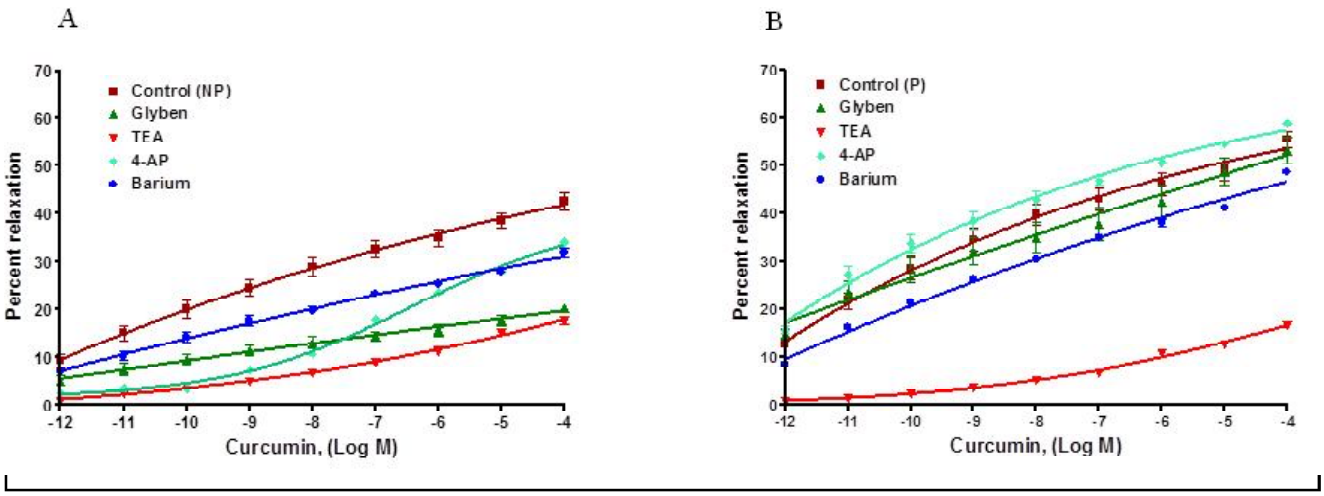

Figure2: Curcumin (1 pM - 100 μM)-induced concentration related response curve elicited on PE-precontracted MUA rings either in absence (control) or in presence of Glibenclamide (Glyben, 1 μM) or TEA (10 mM) or 4-AP (10 μM) or barium (10 μM) in MUA ring of A) NP *Ch* and B) P *Ch*.

3.2 Effect of K^+ channel blockers on NCVR

Table 2 presents the percent mean maximal vasorelaxation (R_{max}) and pIC_{50} of nanocurcumin in absence or presence of K^+ channel blockers in MUA of NP and P *Ch*. Representative raw trace shows effect of nanocurcumin (1pM-100 μM) on PE (10 μM)-induced contraction in absence (control) or in presence of Glibenclamide (glyben,1 μM) or TEA (10 mM) in MUA ring of NP and P *Ch* groups at Figures (3A, 3C and 3E) and Figures (3B, 3D and 3F), respectively.

NCVR curve (R_{max} 40.36 ± 2.38%; pIC_{50} 8.83 ± 0.12) was shifted to right with significant (p<0.001) decrease in R_{Bmax} (32.01 ± 1.57%) and non-significant decrease in pIC_{50} (6.82 ± 1.3) in presence of Glibenclamide (1 μM), significant (p<0.001) decrease in R_{Bmax} (25.82 ± 0.62%) and pIC_{50} (8.0 ± 0.13) in presence of TEA (10 mM), significant (p<0.001) decrease in R_{Bmax} (21.75 ± 0.16%) and significant (p<0.001) decrease in pIC_{50} (6.52 ± 0.11) in presence of 4-AP (10 μM), significant (p<0.05) decrease in R_{Bmax} (27.60 ± 0.55 %) and non-significant increase in pIC_{50} (9.14 ± 0.10) in presence of barium (10 μM) (Figure 4A). In MUA of P *Ch* NCVR curve (R_{max} 44.09 ± 3.41%; pIC_{50} 6.05 ± 0.09) shifted to left with significant (p<0.05) increase in R_{Bmax} (56.42 ± 2.65%) and significant (p<0.001) increase pIC_{50} (9.39 ± 0.14) in presence of glibenclamide, shifted to right with significant (p<0.05) decrease in R_{Bmax} (30.70 ± 0.68%) and significant (p<0.001) increase pIC_{50} (7.30 ± 0.16) in presence of TEA, significant decrease (p<0.05) in R_{Bmax} (34.38 ± 0.86%) and significant (p<0.001) increase in pIC_{50} (8.90 ± 0.15) in presence of 4-AP, non-significant decrease in R_{Bmax} (36.96 ± 1.31%) and significant (p<0.001) increase in pIC_{50} (8.80 ± 0.14) in presence of barium. (Figure 4B). Percent relaxation induced by curcumin and nanocurcumin in presence of K^+ channel blockers shown in Figure 5.

Table 2: R_{max} and pIC_{50} of nanocurcumin (NC) (1pM-100 μM)-induced vasorelaxation on PE (10 μM) precontracted MUA rings in absence (control) or presence of glibenclamide (1 μM) or tetraethylammonium (TEA, 10 mM) or 4-aminopyridine, (4-AP, 10 μM) or barium (10 μM) in MUA of NP or P *Ch*. The values are expressed as Mean ± SEM, N = total numbr of MUA rings used in the experiments

Treatment	N Value		R_{max}/RB_{max} (%)		pIC_{50}	
	NP	P	NP	P	NP	P
Control (NC)	10	10	40.36 ± 2.38	44.09 ± 3.41	8.83 ± 0.12	6.05 ± 0.09
Glibenclamide	6	6	32.01 ± 1.57	56.42 ± 2.65[b]	6.82 ± 1.30	9.39 ± 0.14[a]
TEA	6	6	24.82 ± 0.62[a]	30.70 ± 0.68[b]	8.0 ± 0.13[a]	7.30 ± 0.16[a]
4-AP	6	6	21.75 ± 0.16[a]	34.38 ± 0.86[b]	6.52 ± 0.11[a]	8.90 ± 0.15[a]
Barium	6	6	27.60 ± 0.55[b]	36.96 ± 1.31	9.14 ± 0.10	8.80 ± 0.14[a]

[a](p<0.001), [b](p<0.05) represents level of significance between the rows within each column (with respective controls)

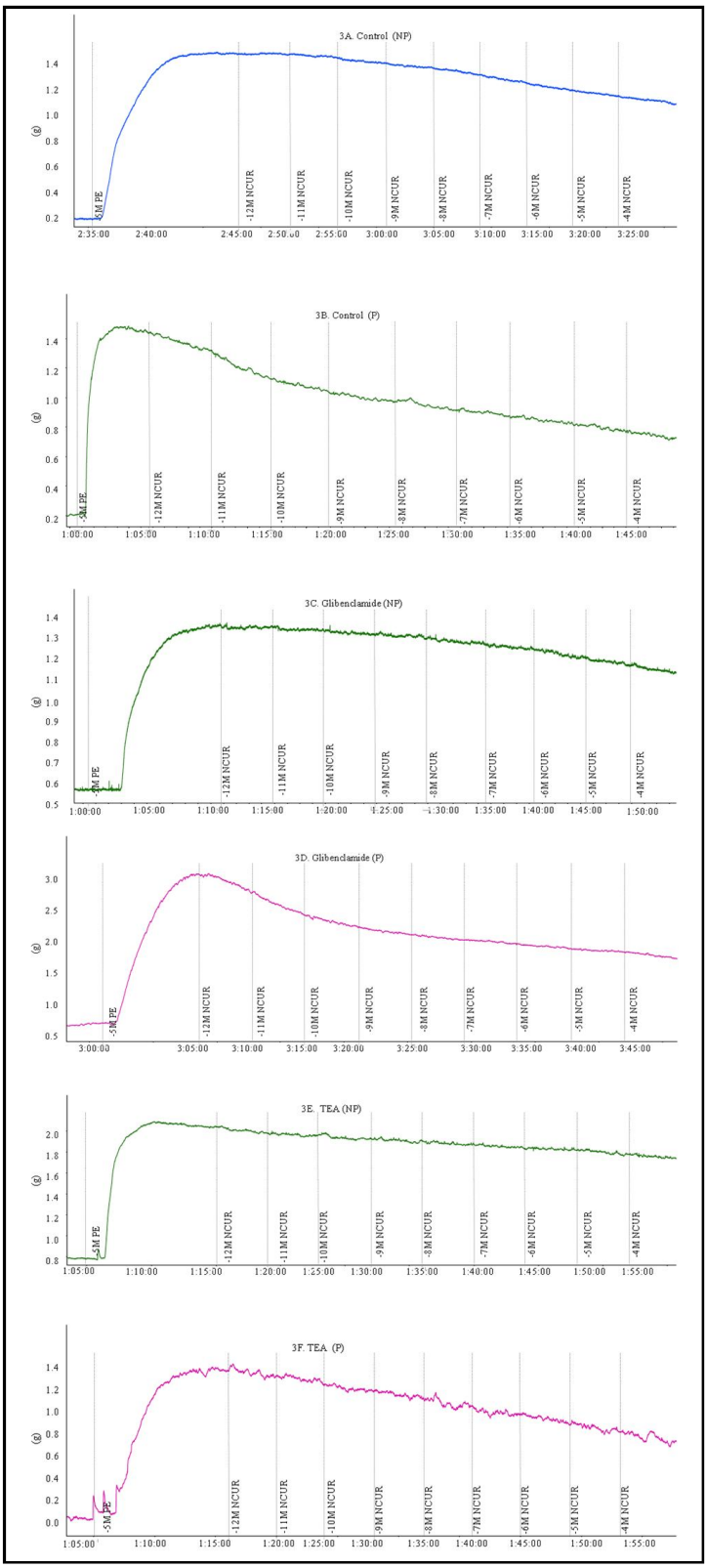

Figure 3: Representative raw trace showing effect of Nanocurcumin (1 pM - 100 µM) on PE (10 µM) - induced contraction in absence (Control) or presence of glibenclamide (Glyben, 1µM) or TEA (10 mM) in MUA ring of NP and P *Ch*. Vasoralxation effect of nanocurcumin (control) and its ameliorative effect by glibenclamide, TEA is shown at Figure 3A, 3C, 3E in NP and Figure 3B,3D,3F in P groups.

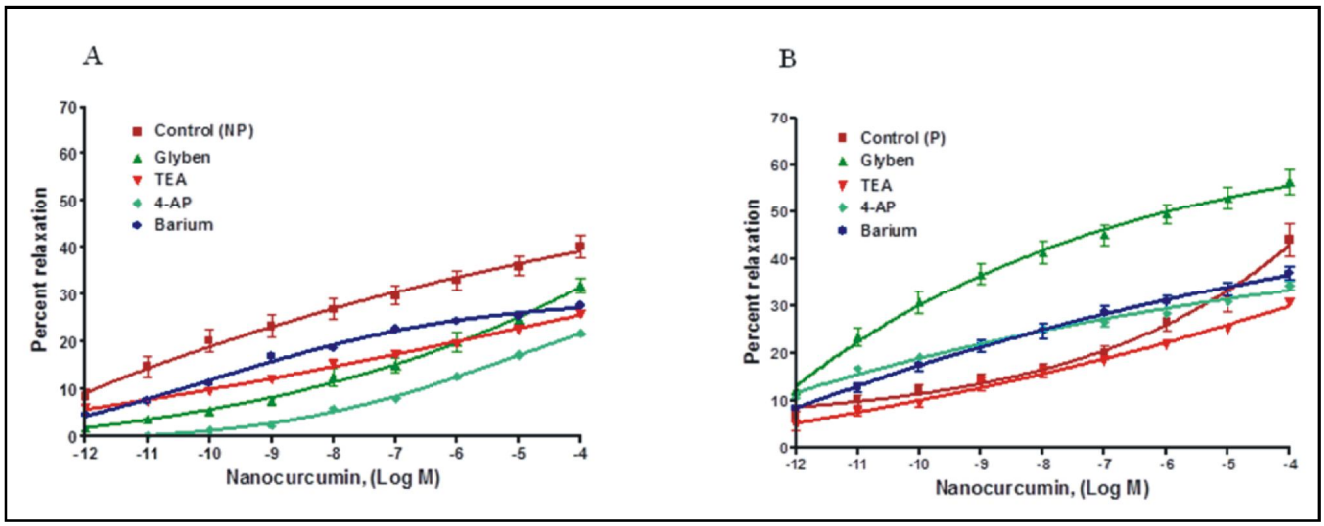

Figure 4: Nanocurcumin (1 pM - 100 μM)-induced concentration related response curve elicited on PE-precontracted MUA rings either in absence (control) or in presence of glibenclamide (Glyben, 1 μM) or TEA (10mM) or 4-AP (10 μM) or barium (10 μM) in MUA ring of A) NP *Ch* and B) P *Ch*.

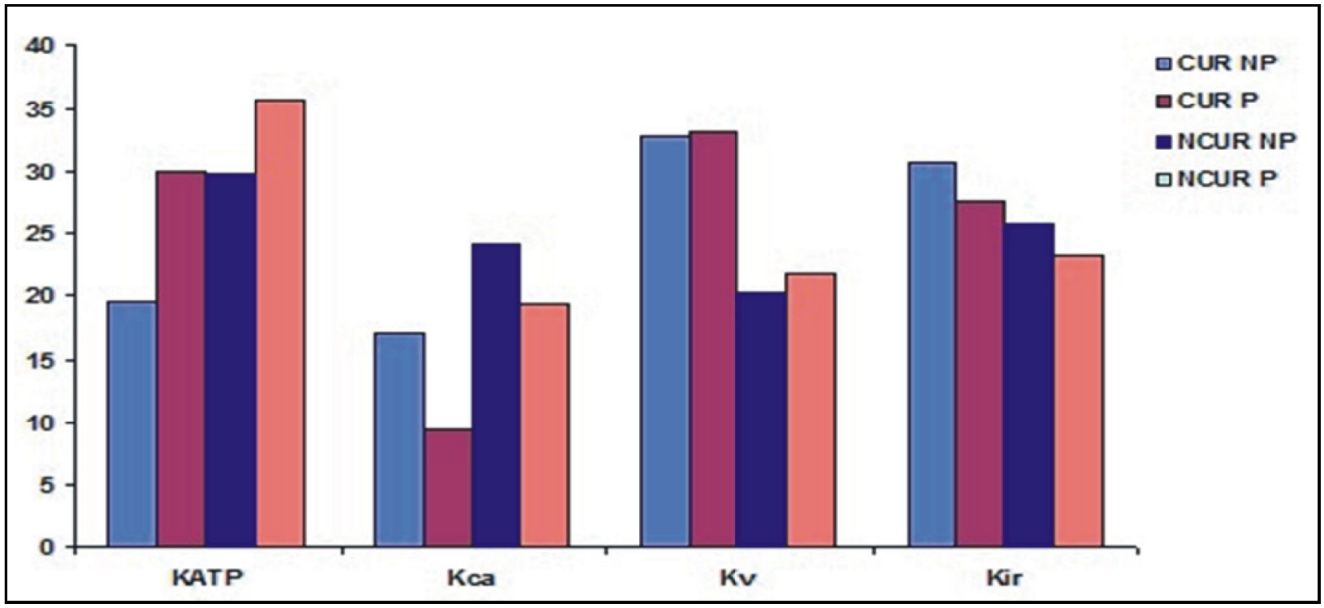

Figure 5: Bar diagram showing differential activation of K_{Ca}, K_V,K_{ATP} and K_{ir} channels by curcumin and nanocurcumin data represent the mean maximal per cent of inhibition of curcumin and nanocurcumin-induced vasorelaxation by glibenclamide (glyben, 1 μM) or TEA (10 mM) or 4-AP (10 μM) or barium (10 μM) in MUA ring of NP and P *Ch*.

4. Discusion

In the present study, the attenuation of CVR/NCVR was examined in presence of glibenclamide (K_{ATP} channel blocker), 4-aminopyridine (K_V channel blocker), TEA (BK_{Ca} channel blocker) and $BaCl_2$ (K_{ir} channel blocker) to assess the sensitivity of different type of K^+ channels to curcumin and nanocurcumin in uterine artery. The significant findings are: (i) R_{max} of curcumin obtained from CVR curve elicited in MUA rings was greater in P *Ch* (55.49%) than that of NP *Ch* (42.58%). Glibenclamide, TEA, 4-AP, and barium reduced the maximal CVR to 20.31%, 17.68%, 34.13%, and 31.94% in NP. The maximal CVR was attenuated to 16.67% and 48.64% by TEA and Ba^{2+} but almost unchanged by glibenclamide and 4-AP in MUA rings of P *Ch*, (ii) The maximal vasorelaxation obtained from NCVR curve elicited in MUA rings was attenuated to 44.09% in P and to 40.36% in NP *Ch*. Glibenclamide, TEA, 4-AP and barium reduced the maximal NCVR to 32.01%, 25.82%, 21.75% and 27.60% in NP. The maximal NCVR was attenuated to 30.70%, 34.38% and 36.96% by TEA, 4-AP, Ba^{2+} but glibenclamide augmented to 56.42% in MUA of P *Ch*.

According to recent estimates from the World Health Organization, hypertension is responsible for the premature death of 7.5 million people each year, attribute for approximately 13% of total deaths

(Zhuang *et al*., 2016). To overcome the pathological situations, several drugs like methyl dopa, hydralazine, β-blockers and diuretics are used by trial and error method, but nothing is found effective. Despite various therapeutic interventions to control or to treat hypertension, no individual drug/agent entirely serves the purpose. Middle uterine artery (MUA) supplies circulation to uterus and maintain the uterine tonicity, it undergoes remodelling during pregnancy to increase blood supply to fetus. Any changes occurs in vasoreactivity of MUA could affect the stress cycle, oestrus, conception and also maintenance of pregnancy. In view of the pivotal role of MUA, it is essential to know the mechanism of vasorelaxation. Owing to the curcumin importance in traditional/folk medicine (Aggarwal and Harikumar, 2009) and its ability to induce vasorelaxation motivated us to investigate the importance of curcumin/nanocurcumin in ameliorating hypertension during pregnancy.

Activation of K^+ channels causes a vasodilation and inhibition results in vasoconstriction in arterial smooth muscles. Increased levels of sex steroids attenuate PKC-mediated signaling in uterine vasculature, thereby causing differential upregulation of K^+ channels activity contribute to the decreased uterine vascular tone and increased uterine blood flow in pregnancy (Xiao *et al*., 2006; Zhu *et al*., 2013). Several observations in different vascular beds showed that curcumin vasorelaxtion involves activation of different K^+ channels. In whole cell patch-clamp experiments on bovine adrenal zona fasciculate (AZF) cells, it was found that curcumin reversibly inhibited the Kv1.4 K^+ current with an IC_{50} of 4.4 μM and a Hill coefficient of 2.32 (Liu *et al*., 2006). In rabbit, arterial smooth muscle cells and rabbit isolated coronary smooth muscle cells, Curcumin inhibition was significantly enhanced by repeated depolarization and that was due to inhibitory effect on voltage-dependent K^+ channels (K_v) (Hong *et al*., 2013). Curcumin-mediated gastric protective effect in the indomethacin-induced gastric injury in rat model indicated that curcumin activates NO/cGMP/K_{ATP} pathway during its gastro protective action (Díaz-Triste *et al*., 2014). Curcumin relaxed both cholecystokinin octapeptide-(CCK) and KCl-induced tension of guinea pig gallbladder strips in a concentration dependent manner and the use of TEA (5 μM), caused a significant ($p < 0.01$) decrease in the amount of curcumin-induced relaxation. This suggested that curcumin-induced relaxation is mediated by multiple signaling pathways including the PKC second messenger system, inhibiting L-type Ca^{2+} channels and opening of K^+ channels (Kline and Karpinski, 2015). The vasodilation effect of Curcumin in rat superior mesenteric artery is endothelium-dependent, involving endothelium-dependent prostanoid pathway, endothelium-independent pathway and blockade of Ca^{2+} influx (Zhang *et al*., 2017).

In our present study, K^+ channels blockers, glibenclamide, TEA, 4-AP and barium reduced the maximal CVR to 20.31%, 17.68%, 34.13% and 31.94% in NP *Ch*. The maximal CVR was attenuated to 16.67%, 48.64% and 25.96% by TEA and Ba^{2+} but almost unchanged by glibenclamide and 4-AP in MUA rings of P *Ch*. This shows that curcumin cause hyperpolarizing effect in VSMCs of MUA through differential activation of different K^+ channels, *i.e*. $K_{Ca} > K_{ATP} > K_{ir} > K_v$ in NP and $K_{Ca} > K_{ir}$ in P *Ch*. It is accepted that activation of cGMP and cAMP may cause opening of several K^+ channels that induce hyperpolarization and vasorelaxation in several vascular beds. So, it could be possible that curcumin either directly or indirectly augments the functions of cGMP and cAMP that in turn opens different K^+ channels resulting in hyperpolarization or directly opens the different K^+ channels to cause hyperpolarization and vasorelaxation of VSMCs. In view of an identical and greater sensitivity of K_{Ca} to TEA in inhibiting the CVR in MUA of NP and P *Ch* it appears that the later mechanism is highly appropriate in explaining the vasorelaxtion effect of curcumin. In conclusion, curcumin cause direct hyperpolarization and vasorelaxation of VSMCs by opening of K_{Ca}, K_{ATP}, K_{ir} , K_v channels in NP and K_{Ca}, K_{ir} channels in P *Ch*.

TEA, 4-AP and barium significantly reduced the maximal NCVR to 25.82%, 21.75% and 27.60% in MUA of NP *Ch*. In contrast, glibenclamide non-significantly reduced the NCVR. The present observation clearly demonstrates that nanocurcumin almost identically activates the BK_{Ca}, K_V and K_{ir} in the VSMC membrane that in turn causes hyperpolarization and vasorelaxation. In MUA of P *Ch*, TEA, and 4-AP but not barium significantly attenuated the R_{Bmax} of NCVR curve to 30.70% and 34.38%, In contrast, glibenclamide significantly augmented it to 56.42%. These findings clearly demonstrate that NC activates opening of BK_{Ca} and K_V channels that leads VSMC membrane hyperpolarization and vasorelaxation. The augmentation of NCVR after blocking of K_{ATP} channels could be occurring due to reduction of binding of glibenclamide to K_{ATP} channels by NC.

5. Conclusion

i. Curcumin and nanocurcumin showed differential sensitivity to different K^+ channels, ii. Curcumin 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*. iii. Nanocurcumin activates the K_V, K_{Ca}, K_{ir} channels in NP *Ch*, and opens K_{Ca} and K_V channels to cause hyperpolarization in P *Ch*, iv. Curcumin/ nanocurcumin targeting K^+ channels will help to develop therapeutic strategies to prevent pregnancy-induced complications such as preeclampsia.

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Conflict of interest

We declare that we have no conflict of interest.

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