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Vasorelaxation to Eugenol, Curcumin and Nanocurcumin mediated by differential augmentation of Na^+, K^+-ATPase activity in middle uterine artery of *Capra hircus*

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Sodium-potassium ATPase (Na, K-ATPase) is an integral protein universally distributed in the plasma membrane of all eukaryotic cells. It regulates trans-epithelial sodium ion transport, muscle contraction, nerve excitability, secretary functions and intracellular signaling. We investigated the mechanism of vasorelaxation effect of Eugenol, Curcumin and Nanocurcumin in the middle uterine artery (MUA) of goat to evaluate their possible therapeutic potential in hypertension during pregnancy or pre-eclampsia. The UA rings were isometrically contracted by K^+-free MKHS to plateau and relaxed with KCl (10 µM-10 mM) following standard protocol. The K^+ -vasorelaxation response (VR) was taken either in presence of ouabain with or without Eugenol/Curcumin/Nanocurcumin (10 µM). R_{max} (maximum % vasorelaxation) of K^+-induced VR-curve elicited in UA rings was reduced (from 6.90% and 26.55%) to 4-5% in presence of Ouabain in both NP and P goats. R_{max} obtained from KVR curve elicited in UA rings were augmented in presence of Eugenol (NP: 16.22%, P: 42.67%) or Curcumin (NP:21.23%, P:35.49%) or Nanocurcumin (NP:6.91%, P:34.88%). K^+ vasorelaxation was inhibited in presence of Ouabain and Eugenol/ Curcumin/Nanocurcumin (R_{max}: 6.11%, 9.18%, 4.11% in non-P, respectively) and (R_{max}: 5.40%, 3.39%, 6.00% in P, respectively). The results indicated that (i) there is increased function of α_1- Na^+, K^+-ATPase in UA of P goats (ii) Eugenol, Curcumin and Nano-curcumin differentially augmented the function and expression of α_1 isoenzymes of sodium pump in UA of non-P and P goats. Thus, the polyphenols potentially activate α_1 isoenzymes of Na^+-K^+-ATPase and could possibly be useful as therapeutic in hypertension during pregnancy.

Keywords: Curcumin, Eugenol, Goat uterine artery, Na^+, K^+-ATPase, Nanocurcumin

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Na^+, K^+-ATPase is an electrogenic, heterodimeric transmembrane protein that regulates sodium pumps and cell signalling[1]. It plays a vital role in generation of electrochemical gradient through the cell membrane by pumping out 3 Na^+ and influx of 2 K^+ ions besides maintenance of cellular homeostasis[2]. The vasorelaxation induced by vascular sodium pump plays a pivotal role in regulation of membrane potential and smooth muscle tone in different vascular tissues[3]. The regulation of this enzyme functional activity and expression is substantially influenced by gender[4], species[5], sex and tissue location[6,7]. The endothelium dependent vascular sodium pump has been reported in human placental vessels[8], rabbit aorta[9], mice femoral artery[10] and rat aorta[11], goat mesenteric artery[12] and endothelium independent in goat ruminal artery[13].

Nutraceuticals such as Eugenol and Curcumin, demonstrated to be useful in several health disorders including hypertension[14-17]. Due to their pleotropic action, several cellular and molecular targets have been proposed in variety of experimental models and cell lines. During pregnancy hypertension is a major concern in certain conditions like preeclampsia. Eugenol, Curcumin and Nanocurcumin could be the safest nutraceuticals to induce uterine artery vasodilation to facilitate maternal blood flow to foetus thus assist in maintenance of normal maternal blood pressure. With this hypothesis we examined their capability to increase the vascular Na^+ pump activity, an important mediator of vasorelaxation. Eugenol is the active component of cloves (*Syzigium aromaticum*) and curcumin is obtained from turmeric (*Curcuma longa*) (Fig. 1 & Fig. 2). Eugenol is commonly used as flavouring agent, in preparation of cosmetics and as anaesthetic in dentistry.

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It is widely used in folk medicine as analgesic, antiseptic, antispasmodic, antineuralgic, carminative, anti-inflammatory, disinfectant, insecticide, stimulant, stomachic, uterine and tonic[14-15]. Eugenol has been reported as antispasmodic and able to induce smooth muscle relaxation and vasorelaxation effects in diverse vascular beds[18]. Analgesic activity of Eugenol in dental medicine is mostly owing to inhibition of ion channels (Na^+, K^+ and Ca^{2+})[19]. Eugenol decreased the castor oil - induced diarrhoea, Prostaglandin E_2 (PGE_2) induced fluid accumulation in intestines and tone of ileal smooth muscles and myometrium in rats[20]. Myogenic antispasmodic effect of Eugenol in rat airway smooth muscles contracted by electrical field stimulation (EFS) suggested that the mechanisms involved may be due to inhibition of voltage/receptor operated Ca^{2+} channels and IP_3-receptor in the sarcoplasmic reticulum[21]. Aqueous extracts of cloves (0.0625%) significantly decreased (95%) the normal activity of sodium-potassium ATPase in rat liver[22].

Curcumin (diferuloylmethane) is a dietary polyphenolic, elicit plethora of health benefits, and alleviates various disease conditions such as malignant, neurodegenerative, cardiovascular diseases, inflammatory and neurological disorders. It is also used as an alternative therapy for uterine leiomyoma[16,17]. In these disease conditions, curcumin nanoparticles expressed greater therapeutic advantage over the native curcumin[23]. Modulatory role of curcumin on sodium – potassium ATPase was described in human erythrocytes[24], shark rectal glands, pig kidney[25] and brain microsomes in rat[26].

Poor solubility, low systemic bioavailability and short half-life are the major problems associated in hindering the use of Curcumin in therapeutics. Synthesis of nano forms of curcumin emerged as an alternative to native curcumin. Studies have shown that nanocurcumin improved the bioavailability of the drug at the target site by enhanced permeability and retention (EPR) effect[27]. Recent reports suggested that nanocurcumin act as anticancer agent against neoplasticity in different organs[28]. It may be a promising lead for reducing hypertension. Administration of eugenol/curcumin in proper dose of can reduce hypertension during pregnancy and this could be one of the potential adjuncts for treatment of pre-eclampsia.

Information on the relative action of nutraceuticals on activation of Na^+, K^+-ATPase is almost lacking in any uterine artery. Therefore, the goal was to evaluate (i) the differences in KCl-induced vasorelaxation response (KVR) regulated by ouabain sensitive sodium pump and (ii) the effect of different nutraceuticals (Eugenol, Curcumin and Nanocurcumin) on the augmentation of KVR if any in UA of non-P and P goat. Our findings would provide first-hand information on the relative potential of Eugenol/Curcumin/Nanocurcumin- causing vasorelaxation in UA of NP and P and their influence on regulation of function of vascular Na^+, K^+-ATPase.

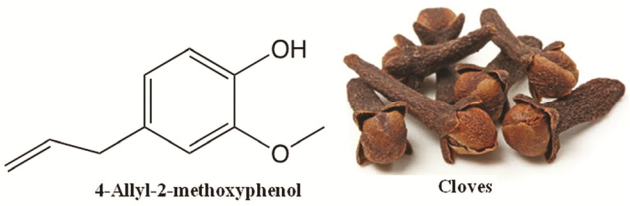

Fig. 1 — Structure and source of Eugenol

Materials and Methods

Ethical guidelines

Ex vivo experimental protocol was approved from the Institutional Animal Ethical Committee with file no. 1586 (6)/16.

Tissue preparation and isometric tension recording

Non pregnant and pregnant uteri are collected from slaughter house in Modified Krebs-Henseleit Saline

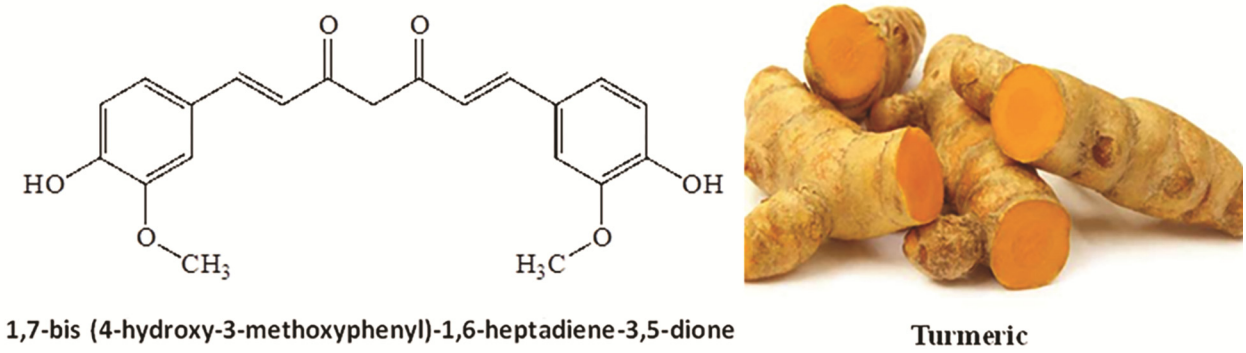

Fig. 2 — Structure and source of Curcumin

(MKHS) solution (mM): $NaHCO_3$ 11.9, NaCl 118, dextrose 11.1, $MgSO_4$ 1.2, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5 and pH 7.40. In the laboratory, the secondary branch of UA was cleaned carefully and dissected into circular arterial rings (1.5-2 mm). Individual arterial rings were clamped to an L-shaped stainless steel wire hooks and mounted in a thermostatically controlled (37.0±0.5ºC) 20 mL capacity tissue bath (Pan Lab) with basal tension of 1.5 g. The bathing solution in the tissue bath was supplied with continuous bubbles of carbogenic mixture (95% O_2+5% CO_2). The arterial rings were super fused continuously with MKHS and bathing solution was replced every 15 min. The variation in net isometric contraction was recorded using high sensitive force transducer connected to 8/32 amplifier and analysed using LabChart 7.1.3 software.

Experiments with Potassium-free modified KHS

To assess the role of sodium pump in vascular endothelium, the arterial rings were equilibrated in the Pss for 1 hour and exposed to 5-HT (1.0 µM) to obtain a contraction. ACh (1µM) was added to bath to observe relaxation in UA rings pre-contracted with 5-HT. The UA rings were then exposed to Pss for 30 min and further the bath solution was replaced with to potassium free MKHS solution to obtain a sustained contraction.

Concentration related vasorelaxtion response to Potassium with or without Ouabain (1 µM) and Eugenol/Curcumin/Nanocurcumin (10 µM) in pre-contracted UA rings.

The arterial ring was incubated in K^+-free MKHS solution with or without Ouabain and Eugenol/ Curcumin/Nanocurcumin for 30 min. Subsequently, Potassium chloride was added (10 µM-10 mM) to the bath in a cumulative manner at an interval of 1 min to record the contractile response. The MUA rings were incubated with L-NAME (1 µM) before addition of KCl in order to eliminate the possible release of NO.

Data analysis

The data was indicated as Mean±SEM. The % maximum relaxation (R_{max}/ R_{Bmax}) to Potassium (control/in the presence of other ligands) were calculated from net gram tention. Concentration related vasorelaxation curves were constructed and –logIC_{50}/pIC_{50} was analysed using interactive non-linear regression with the help of Graph Pad Prism 5 Software (USA). Statistical significance was set to $p<0.05$ and <0.001 for all experiments.

Drugs

Acetylcholine chloride, Ouabain and 5-hydroxy tryptamine, Eugenol and Curcumin were purchased from Sigma Chemicals Pvt Ltd. and one of the author Dr. Bimal P Mohanty gifted Nanocurcumin. Solutions were prepared using triple distilled water. Eugenol (99% purity) stock solution was prepared in dimethyl sulfoxide and Curcumin (>80% purity) prepared in 0.5N NaOH and phosphate buffered saline.

Results

Vasorelaxation to ACh in 5-HT (0.1 M) pre-contracted MUA rings.

After 1 hr incubation with MKHS solution UA ring was contracted on exposure to 5-HT (0.1 µM). Further addition of ACh (1 µM) to the bath, the 5-HT-induced sustained contraction was inhibited the arterial contraction suggesting the presence of functional endothelium.

Effect of Ouabain (1µM) on KCl -induced vasorelaxation in MUA rings

Table 1 presents R_{max} and pIC_{50} of KCl-induced CRC in absence or presence of ouabain, eugenol, curcumin,

Table 1 — The R_{max} (%)and pIC_{50} obtained from of K^+ (10µM -10mM) vasorelaxation response curve (control). The changes in R_{Bmax} and pIC_{50} of KVR curve with Ouabain, (1µM) or Eugenol or Ouabain+Eugenol or Curcumin or Ouabain+Curcumin or Nanocurcumin or Ouabain+Nanocurcumin were listed. Each data level shows the Mean ±SEM, N= total number of data.

TREATMENT	N		R_{max}/ R_{Bmax} (%)		-LOG IC_{50} (pIC_{50})	
	NP	P	NP	P	NP	P
KCl Control	6	6	6.90±0.55	26.58±2.07	3.47±0.06	3.79±0.06
Ouabain	6	6	4.21±0.36[b]	4.87±0.55[a]	3.92±0.07[a]	3.81±0.04
Eugenol	6	6	16.22±1.12[a]	42.67±0.55[a]	3.17±0.09[b]	2.59±0.09[a]
Ouabain+Eugenol	6	6	6.11±0.45	5.40±0.76[a]	3.83±0.04[a]	4.47±0.10[a]
Curcumin	6	6	21.23±0.22[a]	35.49±0.52[b]	2.60±0.06[a]	2.0±0.02[a]
Ouabain+Curcumin	6	6	9.18±1.14	3.39±0.10[a]	3.94±0.05[a]	3.87±0.05
Nanocurcumin	6	6	6.91±0.35	34.58±0.25[b]	1.53±0.29[a]	2.43±0.08[a]
Ouabain+Nanocurcumin	6	6	4.11±0.05[a]	6.00±0.66[a]	2.94±0.04[a]	2.57±0.11[a]

[a]($p<0.001$), [b]($p<0.05$) shows values are significantly different from that of the control values within a particular column.

Nanocurcumin or in combination with ouabain in UA of non-P and P goat. Representative raw traces showing effect of eugenol, curcumin and nanocurcumin on KCl-induced contraction in Uterine arterial ring of both groups have been presented in Figure 3A-D. Addition of K^+-free solution to the bathing solution resulted a rise in basal tone in about 30 min and, subsequently, a sustained contraction was noticed in 3 min. Cumulative addition of KCl (10 µM–10 mM) inhibited sustained contraction in ED+MUA rings of NP and P *Ch*. KCl (10 µM–10 mM) caused concentration related vasorelaxation (R_{max} 6.90±0.55, pIC_{50} 3.47±0.06%) in K^+ free PSS precontracted MUA rings of NP *Ch*. Ouabain inhibited the vasorelaxation to KCl with rightward shift of VR curve by significant ($p<0.05$) decrease in R_{Bmax} (4.21±0.36%) and significant increase ($p<0.001$) in pIC_{50} (3.92±0.07). In uterine artery of P goat, KCl (10 µM–10 mM) caused concentration related vasorelaxation (R_{max} 26.58±2.07%, pIC_{50} 3.79±0.06) in K^+ free PSS precontracted rings. In presence of Ouabain, there was rightward shift of KVR curve with significant ($p<0.001$) reduction of R_{Bmax} (4.87±0.55%) and non significant enhancement of pIC_{50} (3.81±0.04).

Effect of Eugenol (10 µM) or Ouabain (1 µM) and Eugenol (10 µM) on KCl -induced vasorelaxation

In presence of Eugenol (10 µM), VR to KCl was augmented with significant ($p<0.001$) increase in R_{Bmax} (16.22±1.12%) and ($p<0.05$) decrease in pIC_{50} (3.17±0.09). In the presence of both Ouabain (1 µM) and Eugenol (10 µM), R_{Bmax} and pIC_{50} obtained from KVR curve were decreased (6.11±0.45%) and increased (3.83±0.04), respectively in MUA of NP *Ch* (Fig. 4A). In MUA of P *Ch*, as observed from the KVR curve there was significant ($p<0.001$) increase in R_{Bmax} (42.67±0.55) and decrease in pIC_{50} (2.59±0.09) in presence Eugenol, decrease in R_{Bmax} (5.40±0.76%) and increase in pIC_{50} (4.47±0.10) in presence of both Ouabain and Eugenol as compared to the control (Fig. 4B).

Effect of Curcumin (10 µM) or Ouabain (1 µM) and Curcumin (10 µM) on KCl -induced vasorelaxation in MUA rings

In non-pregnant goat, in presence of curcumin (10 µM), there was rightward shift of KVR curve with significant ($p<0.001$) increase in R_{Bmax} (21.23±0.22%) and decrease in pIC_{50} (2.60±0.06), in presence of both ouabain (1 µM) and curcumin (10 µM) a non-significant decrease in R_{Bmax} (9.18±1.14%) and significant ($p<0.001$) augmentation in pIC_{50} (3.94±0.05) as compared to KVR (control) (Fig. 5A). In uterine artery of P goat, the KVR was potentiated with significant ($p<0.05$) increase in R_{Bmax} (35.49±0.52) and decrease in pIC_{50} (2.0±0.02) in presence curcumin, was inhibited with significant ($p<0.001$) decrease in R_{Bmax} (3.39±0.10%) and non-

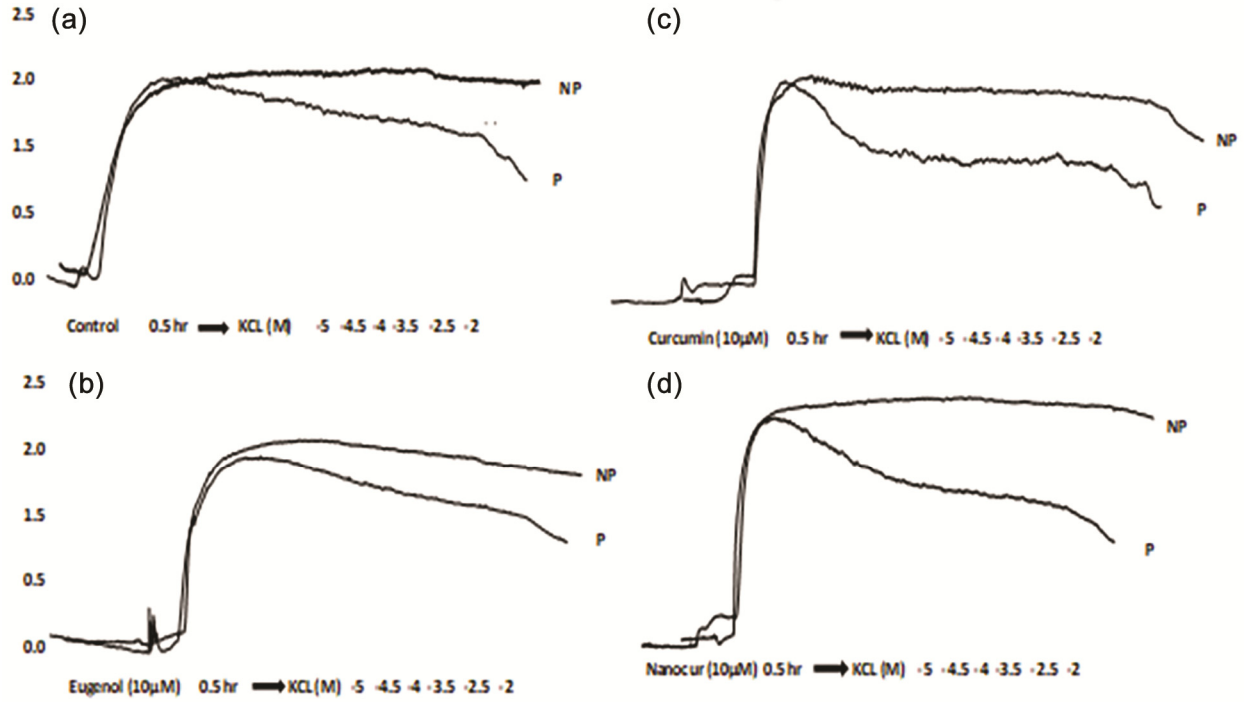

Fig. 3 — Representative raw traces showing effect in absence (Control, 1A) or in presence of 10 µM Eugenol (1B), Curcumin (1C) and Nanocurcumin (1D) on KCl (10 µM-10 mM)-induced contraction in MUA ring of NP and P *Ch* groups.

significant increase in pIC_{50} (3.87±0.05) with both ouabain and curcumin as compared to the KVR (control) (Fig. 5B).

Effect of Nanocurcumin (10 µM) or Ouabain (1 µM) and Nanocurcumin (10 µM) on KCl -induced vasorelaxation in MUA rings.

In MUA of nonpregnant goat, in presence of Nanocurcumin (10 µM), KVR curve was showed non-significant increase in R_{Bmax} (6.91±0.35%) and significant ($p<0.001$) reduction in pIC_{50} (1.53±0.29). Similarly, in the presence of both ouabain (1 µM) and nanocurcumin (10 µM), it was significantly inhibited with reduction in R_{Bmax} (4.11±0.05%) and pIC_{50} (2.94±0.04) (Fig. 6A). In uterine artery of P goat, the KVR was potentiated with increase in R_{Bmax} (34.58±0.25) and decrease in pIC_{50} (2.43±0.08) in presence nanocurcumin only, but it was significantly inhibited with decrease in R_{Bmax} (6.00±0.66%) and pIC_{50} (2.57±0.11) in presence of both ouabain and nanocurcumin (Fig. 6B).

Discussion

The salient findings are (i) the R_{max} obtained from KCl-response curve (KVR) elicited in uterine artery of non-pregnant (6.9%) and pregnant (26.58%) goat was reduced to 4.21% and 4.87% in presence of Ouabain, (ii) Eugenol, Curcumin and Nanocurcumin augmented the R_{max} of KVR curve to 16.22%, 21.23% and 6.91% in UA of non-P goat and it was reversed to

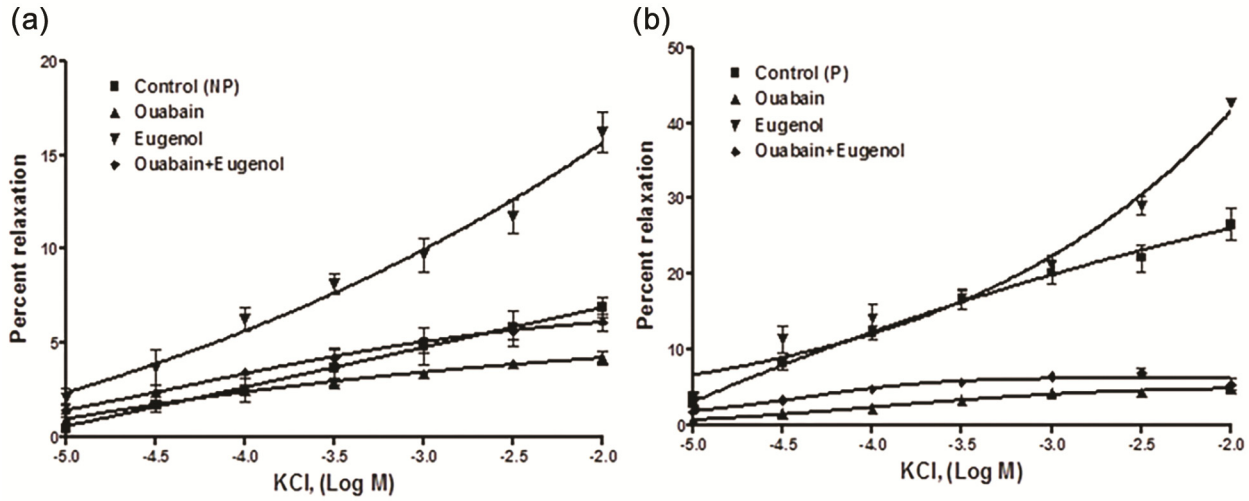

Fig. 4 — The effect of Ouabain (1 µM), Eugenol (10 µM), or in combination of Ouabain (1 µM) and Eugenol (10 µM) on KCl (10 µM-10 mM) vasorelaxation response curve elicited in the utrine arety of A. NP (non-pregnant*)* and B. P (pregnant) goat.

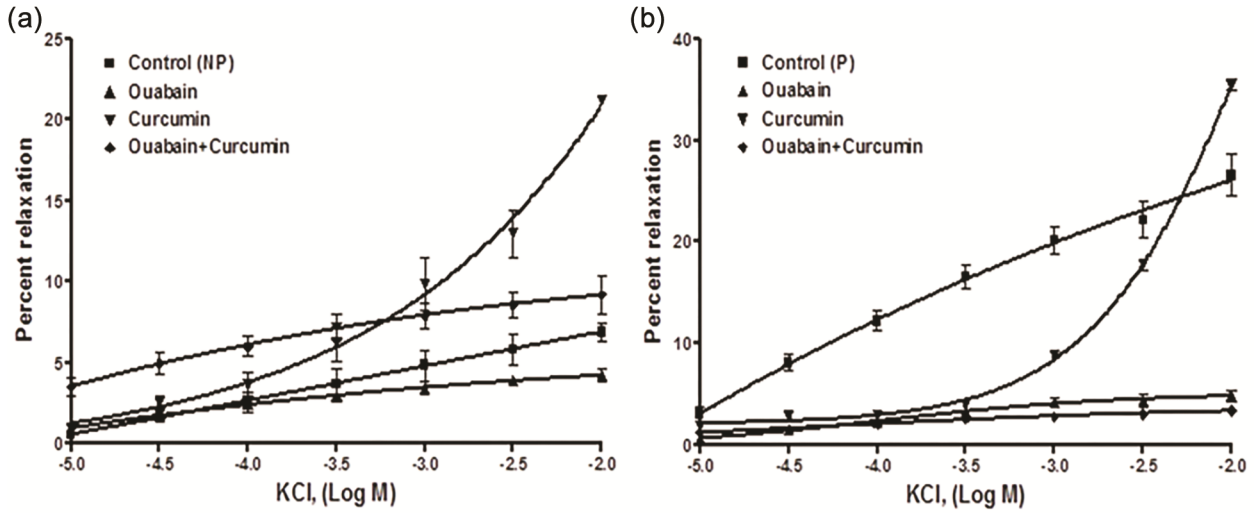

Fig. 5 — KCl (10 µM-10 mM) vasorelaxation response curve constructed with Ouabain (1 µM), Curcumin (10 µM) or in combination of Ouabain (1 µM) and Curcumin (10 µM) in uterine artery of A. NP (nonpregnant) and B. P (pregnant) goat.

6.11%, 9.18% and 4.11% by Ouabain, (iii) these three polyphenols augmented the R_{max} of VR curve of KCl to 42.67%, 35.49% and 34.58% and it was reversed to 5.40%, 3.39% and 6.0% by Ouabain in uterine artery obtained from pregnant goat,suggesting their potential effect in modulating Na^+, K^+-ATPase.

he function of Na^+, K^+-ATPase is critical in perpetuating the potential difference across the cell membrane and interim responsible for vascular tone and BP regulation[3]. In some arterial endothelium this ATPase has been considered as a factor causing cellular hyperpolarization designated as endothelium-derived hyperpolarizing factor (EDHF)[29]. The objective of performing this functional study was to evaluate specifically the role of Ouabain sensitive Na^+, K^+-ATPase enzyme activity in inducing vasorelaxation in uterine artery by lower doses of K^+. The function of this enzyme in maintaining vascular resistance and hypertension in rat renal artery[30], thoracic aorta[2], human placental vessels[8], rabbit aorta[9] and, mice femoral artery[10] has been reported.

Three protein subunits of this -ATPase have been recognized in various tissues such as α, β and γ. In human tissues, four isoforms of α subunits (α1- α4) perform ATP hydrolysis and cation binding[31], whereas three β subunit (β1- β3) maintains the stability and trafficking of the sodium pump[32]. In several mammalian cells, α subunits have greater affinity for inhibition by ouabain with notable exceptions, i.e., guinea pig small intestine[33] and human cardiac muscle[34] in which ouabain-resistant α subunits have been reported. In ovine mesenteric artery, α_1 and α_2 subunits of this enzyme exhibited low and high affinity to Ouabain[12]. In rat vascular tissues, the presence of alpha 1, alpha 2 and alpha 3 subunit has been studied in mesenteric artery[35], aorta myocytes[36] and thoracic, superior mesenteric and tail arteries[5]

Uterine contraction relies on the Na^+, K^+ -ATPase enzyme activity, which generates ionic gradient to induce excitation-contraction coupling. Differences in the alpha and beta isoforms of Na^+, K^+ -ATPase plays a vital role in pregnancy[37]. $Alpha_1$ and $beta_1$ subunits are universally present in tissues and their combination is termed as housekeeping form of Na^+, K^+ -ATPase[38]. Different isoforms of α subunits has been reported to be increased with increase in gestation period in rats. In pregnant rats, expression of Ouabain sensitive isoforms of sodium pump (α_2 & α_3) is increased but not α_1[39].

In our present study, the stability of sustained contraction was checked by 5-HT (0.1 μM) and relaxed with ACh (1 μM). ACh relaxed the 5HT precontracted rings signifying that MUA rings under experimentation has intact endothelium in both NP and P *Ch*. The maximal vasorelaxation obtained from KCl-response curve (KVR) elicited in MUA rings of was 6.9% and 26.58%, respectively. In presence of Ouabain the R_{max} of KCl was reduced to 4.21% and 4.87%. These results clearly show that in pregnant uterine artery the KVR is increased by 4.5 fold and ouabain reverses the R_{max} of KVR in NP and P *Ch* to an identical percent. So our results specifically showed that α_1 subunit isoenzyme of Na^+, K^+-ATPase

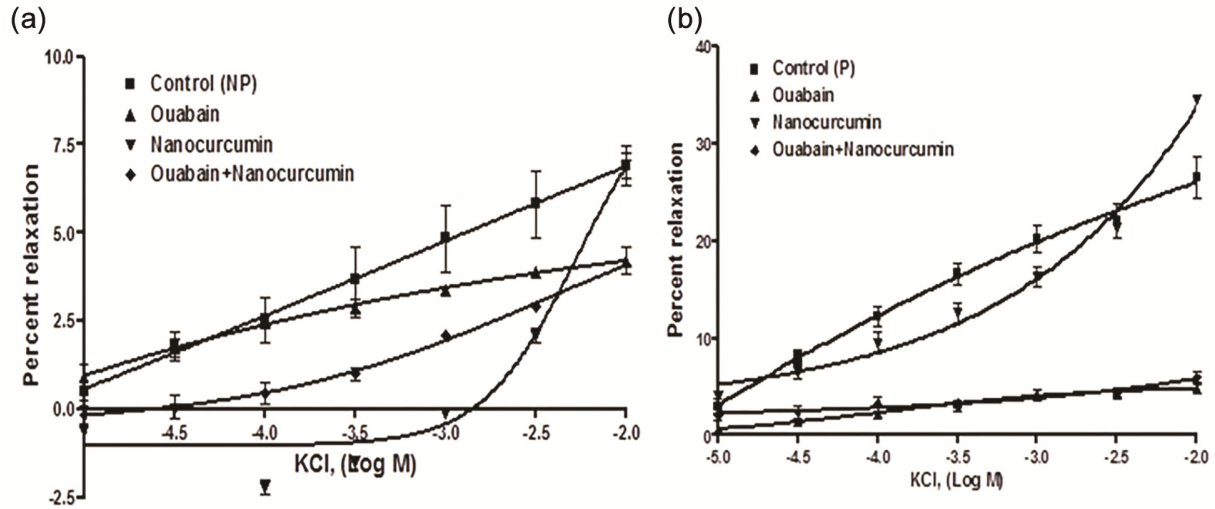

Fig. 6 — The effect of Ouabain (1 μM), Nanocurcumin (10 μM), or in combination of Ouabain (1 μM) and Nanocurcumin (10 μM) on KCl (10 μM-10 mM) vasorelaxtion response curve of uterine artery of **A.** NP and **B**. P goat.

(Ouabain sensitive) has (i) a minimal (<10%) contribution to vasodilation in uterine artery of NP, (ii) a significant increased activity in vasodilation of uterine artery of pregnant goat. The present findings clearly support our previous studies made in other vascular tissues of goat in which Ouabain sensitive Na^+, K^+-ATPase has been demonstrated to contribute differentially to vasorelaxation of ruminal artery[13], mesenteric artery[12] and middle uterine artery[40].

In order to assess the modulatory role of Eugenol on Na^+, K^+-ATPase in uterine vascular beds, KCl-induced vasorelaxation in K^+-free PSS was elicited with Eugenol or Eugenol and Ouabain. Eugenol augmented the R_{max} of KVR curve to16.22% and 42.67% in UA rings of non P and P goat, respectively. Ouabain almost completely reversed these R_{max} of KVR curve to 6.11% and 5.40%. These results clearly indicate that (i) Ouabain sensitive Na^+, K^+-ATPase showed a minimal role in MUA of NP and its activity is increased by more than 4 fold in that of pregnant one, (ii) Eugenol activates the α_1 subunit isoenzyme of Na^+, K^+-ATPase in MUA by 2.4 fold in NP and 1.6 fold in P *Ch* as compared to their respective control (6.9% and 26.58%). These results confirm that Eugenol activates α_1-Na^+, K^+-ATPase that contributes to vasorelaxation in MUA of NP and this is greatly increased in P *Ch*. Contradicting our findings, other findings demonstrated that Eugenol inhibited sodium pump in renal, intestinal, liver of rats and that could be due to reduced intracellular levels of ATP or defects in ion transport causing electrolyte imbalance and derangements in mitochondrial function[22,41]. As on today there are no reports on the vasodilator effect of Eugenol in uterine artery of goat affecting this ATPase function. A higher and moderate augmentation of KCl-induced vasodilation by Eugenol in uterine artery of NP and P goat obtained from our findings clearly demonstrate that Eugenol increases the functional activity of α_1 -Na^+, K^+-ATPase (Ouabain sensitive) in a greater and lower proportion in uterine artery of non-pregnant and pregnant goat, respectively. The possible underlying mechanism involved in augmented vasorelaxation to Eugenol in these tissues could be due to its enhanced phosphorylation of Na^+, K^+-ATPase as there is higher intracellular ATP level in these VSMCs.

Curcumin increased the R_{max} estimated from KVR curve elicited in uterine arterial rings of non-P (6.9%) and pregnant (26.58%) goat to 21.23% in NP and 35.49% in P *Ch.* This demonstrated that Curcumin activates Na^+, K^+-ATPase more potently in P than that of NP *Ch*. Reversal of potentiating effect of Curcumin on KVR by Ouabain indicates that Curcumin augments the vasorelaxation effect via increased function of α_1 is enzyme of Sodium ATPase. Interaction of Curcumin with Sodium pump has been reported to be biphasic in RBCs of human. In these tissues, Curcumin at 10^{-5}M caused inhibition due to interaction with active catalytic sites and this resulted in down regulation of the enzyme. But it, at the concentration range of 10^{-7} - 10^{-8} M modulates RBC cell membrane fluidity and augments the function of Sodium potassium ATPase[24]. Curcumin efficacy and its interaction with sodium pump at protein-lipid interface mainly depends on membrane structure[42]. Curcumin augments Ouabain sensitive Na^+, K^+-ATPase by 3.07 and 1.33 fold in uterine artery of goat. Similarly, Nanocurcumin augmented the R_{max} of KVR curve from 26.58% to 34.58% in the pregnant UA rings but non-significantly in NP *Ch*. Ouabain and Nanocrucumin reduced the R_{max} of KVR to 6% in UA rings of pregnant goat. These findings clearly demonstrate that Nanocurcumin increased Na^+, K^+-ATPase activity by 1.3 fold in P *Ch* but not in NP *Ch*. A greater and lower proportion increase in KCl-induced vasodilation by Curcumin in uterine artery of NP and P *Ch* clearly relates directly to a greater and lower sensitivity of α_1 subunit isoenzyme of this ATPase to Curcumin. An identical sensitivity of α_1 isoform of Sodium pump to Nanocurcumin was also observed in uterine artery of P but not in NP could not be explained with respect to underlying mechanisms as observed with Curcumin.

In conclusion, the vascular resistance of UA of goat is maintained by low level function of Ouabain sensitive Sodium pump in non-pregnancy and this is greatly augmented in pregnancy which could be due to increased expression of α_1 isoenzyme subunits. A greater sensitivity of Curcumin than Eugenol in activating Na^+, K^+-ATPase in non-pregnant rings could be occurring due to differences in the affinity of these polyphenols to interact with active catalytic canter of the enzyme or enhancing the ATP breakdown to increase Na^+, K^+-ATPase function in non-P uterine artery as suggested in erythrocyte membranes. A greater sensitivity of Na^+, K^+-ATPase for Eugenol than Curcumin or Nanocurcumin in pregnant rings may be resulted from increased function of $\alpha_1$isoenzyme subunits of Na^+-K^+-ATPase that is differentially activated by these polyphenols.

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Conflict of Interest

Authors declare no conflict of interest

Author Contributions

Conceptualization: JH, SCP, BPM
Data curation: JH, SCP
Formal analysis: JH
Funding acquisition: JH, SCP
Investigation: JH, SCP
Methodology: JH, SCP
Project Administration: SCP
Resources: JH, SCP, BPM
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