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Tectorigenin induces vasorelaxation in porcine coronary arteries through activation of Kv channels and oestrogen receptor modulation

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Ischaemic heart disease remains a leading cause of mortality worldwide, highlighting the need for new agents that protect vascular function. Tectorigenin, a plant-derived isoflavone, is known for its anti-inflammatory and antioxidant properties, but its direct effects on vascular tone have not been clearly explored. This study investigated the vasorelaxant actions of tectorigenin in endothelium-denuded porcine coronary arteries and examined the mechanisms involved. Using isolated artery rings pre-contracted with a thromboxane A_2 analogue, we found that tectorigenin induced concentration-dependent relaxation with an EC_{50} of approximately 11 μM. Pharmacological inhibition experiments revealed that relaxation at 10–30 μM was significantly reduced by oestrogen receptor antagonists and by 4-aminopyridine, a blocker of voltage-gated potassium channels, and was completely abolished under high-potassium conditions. In contrast, inhibitors targeting neural conduction, nitric oxide synthase, cyclic nucleotide pathways, and other potassium channels had no significant impact. Immunohistochemistry and qPCR analyses indicated predominant ERα expression in the coronary arteries. These findings are consistent with contributions from oestrogen receptors (with ERα predominance) and 4-AP-sensitive Kv channels to the relaxant response; a direct ER-Kv coupling was not established in this ex vivo model. This is the first study to characterise the vascular pharmacology of tectorigenin in a large-animal coronary model. Further in vivo investigations are warranted to assess its potential in managing cardiovascular diseases.

Keywords Tectorigenin, Vasorelaxation, Coronary artery, Kv channels, Oestrogen receptor α, Cardiovascular pharmacology

Abbreviations

4-AP	4-Aminopyridine
BKCa	Large-conductance calcium-activated potassium channel
CTX	ω-Conotoxin GVIA

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cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
DMSO	Dimethyl sulfoxide
EC_{50}	half-maximal effective concentration
ER	Oestrogen receptor
ERα / ERβ	Oestrogen receptor alpha / beta
IHD	Ischaemic heart disease
IbTX	Iberiotoxin
KATP	ATP-sensitive potassium channel
Kv	Voltage-gated potassium channel
L-NNA	Nω-nitro-L-arginine
LAD	Left anterior descending artery
NO	Nitric oxide
KCl	Potassium chloride
PDE	Phosphodiesterase
PKA	Protein kinase A
PKG	Protein kinase G
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
TEA	Tetraethylammonium
TTX	Tetrodotoxin
TXA_2	Thromboxane A_2

Ischaemic heart disease (IHD) is a leading cause of morbidity and mortality worldwide, particularly in ageing populations, and continues to present a major therapeutic challenge despite significant advances in both pharmacological and interventional strategies[1,2]. The complex pathophysiology of IHD involves endothelial dysfunction, vascular inflammation, and impaired coronary perfusion, ultimately leading to myocardial ischaemia and infarction[3–5]. Current pharmacologic therapies—such as β-blockers, calcium channel blockers, nitrates, and antiplatelet agents—primarily aim to reduce myocardial oxygen demand and improve blood flow. However, they fall short of addressing the multifaceted nature of vascular dysfunction[6]. Moreover, interindividual variability in therapeutic response underscores the need for novel vasoprotective agents that engage alternative regulatory mechanisms.

Tectorigenin, an isoflavone derived from several medicinal plant species, has gained attention for its diverse pharmacological properties, including antioxidant, anti-inflammatory, metabolic, and cardiovascular effects[7–9]. In vitro studies have shown that tectorigenin protects endothelial cells under oxidative stress by enhancing cell viability, activating the PI3K/Akt signalling pathway, and suppressing apoptosis in H_2O_2-exposed human umbilical vein endothelial cells (HUVECs)[10]. These findings highlight its potential in preserving endothelial integrity, a critical determinant of vascular function.

Additionally, tectorigenin is known to modulate oestrogen receptors (ERs), interacting with both ERα and ERβ, albeit with differential affinity[11]. Oestrogenic compounds, including phytoestrogens, are known to exert vasodilatory effects via nitric oxide (NO)-dependent and ion channel-mediated mechanisms[12]. These mechanistic insights raise the possibility that tectorigenin may also influence vascular tone, although this has yet to be experimentally confirmed.

Despite these promising properties, no study has systematically investigated whether tectorigenin directly induces vasorelaxation in coronary arteries or the mechanisms involved. Therefore, the present study aimed to determine whether tectorigenin induces vasorelaxation in porcine coronary arteries and to elucidate the underlying molecular pathways. By addressing this knowledge gap, we seek to provide new insights into the vascular pharmacology of tectorigenin and its potential therapeutic relevance in the context of IHD and vascular dysfunction.

Results

Evaluation of tectorigenin's effects on coronary arterial relaxation following U46619-Induced Pre-contractions

Figure 1A presents representative tracings illustrating the vasorelaxant effects of tectorigenin at 10, 30, and 100 μM in porcine coronary artery rings pre-contracted with 100 nM U46619. The corresponding quantification is shown in Fig. 1B, revealing a significant concentration-dependent increase in relaxation. The relaxation rates were 5.47 ± 1.71% (1 μM), 13.26 ± 5.60% (3 μM), 50.72 ± 3.75% (10 μM), 98.13 ± 3.04% (30 μM), 105.17 ± 0.75% (100 μM), and 113.04 ± 2.90% (300 μM). These values were all significantly greater than those observed in the corresponding dimethyl sulfoxide (DMSO) vehicle control group ($p < 0.05$, $n \geq 4$), confirming that the observed effects were not attributable to the solvent.

All experiments included vehicle-treated rings matched for DMSO concentration. Even at the highest tectorigenin dose (300 μM, 3.0% DMSO), vasorelaxation remained significantly greater than that of the vehicle control.

The half-maximal effective concentration (EC_{50}) value of tectorigenin was estimated to be 11.38 ± 0.91 μM using a four-parameter logistic regression model ($R^2 = 0.9990$), indicating an excellent fit to the concentration–response data. Based on this result, 30 μM tectorigenin was selected for subsequent mechanistic investigations of its vasorelaxant effects in porcine coronary arteries.

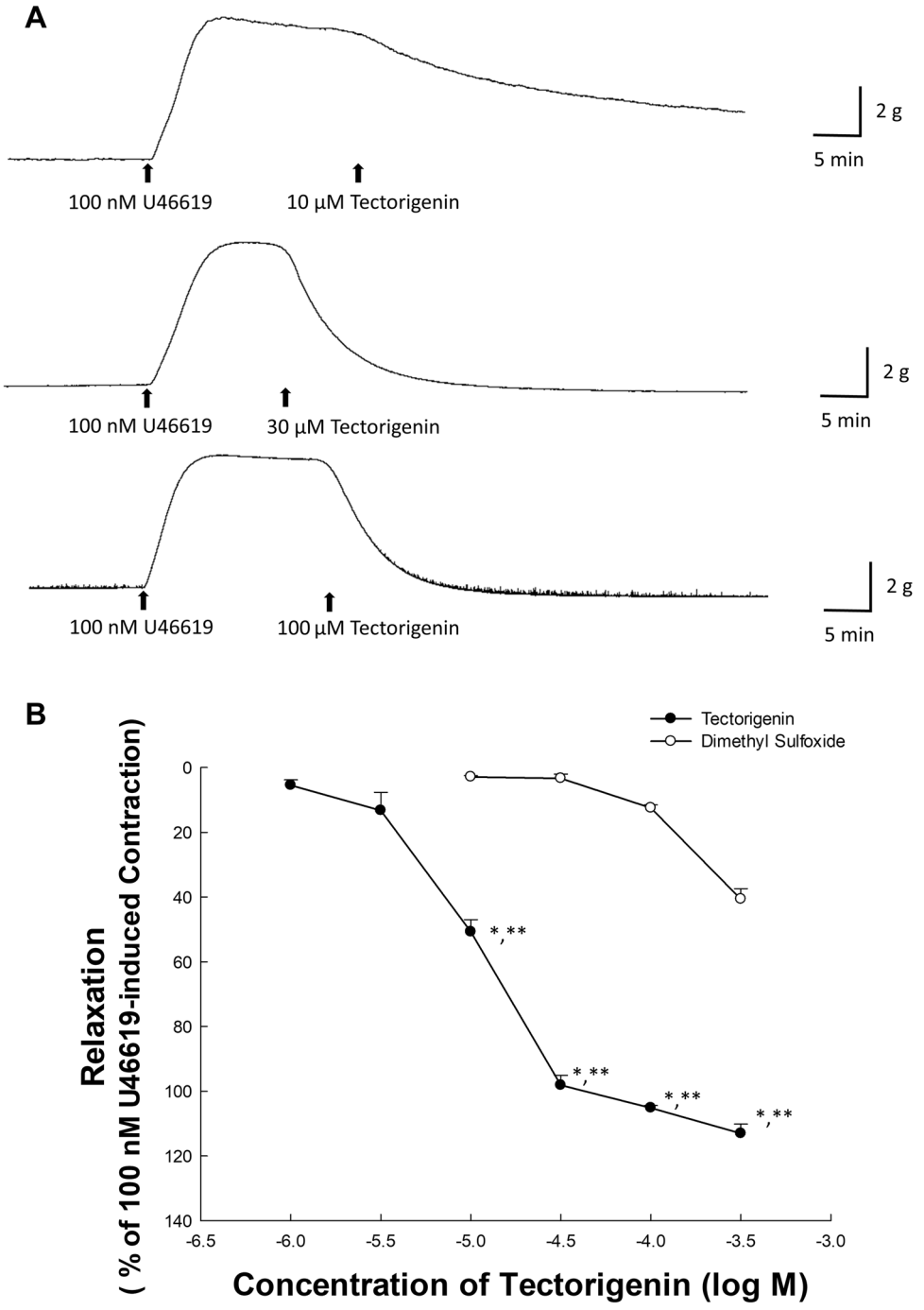

Fig. 1. Tectorigenin-induced vasorelaxation in porcine coronary arteries. (**A**) Representative tracings showing vasorelaxant responses to tectorigenin (10, 30,100 µM) in coronary rings pre-contracted with U46619 (100 nM). Arrows indicate addition of U46619 and tectorigenin. (**B**) Concentration-dependent relaxation versus DMSO control. Relaxation is expressed as % of U46619-induced tone. Data are mean ± SEM ($n \geq 4$). *$p < 0.05$ vs. vehicle; **$p < 0.05$ vs. 10 µM tectorigenin. A 4-parameter logistic curve fit yielded EC_{50} = 11.38 ± 0.91 µM (R^2 = 0.9990).

Neural conduction's influence on Tectorigenin-Induced relaxation in Porcine coronary arteries

U46619 pre-contraction (plateau), normalised to each ring's 60 mM KCl tone, did not differ between the no-inhibitor control and the inhibitor-treated groups at 30 µM (Dunnett vs. control, $p > 0.05$), shown in Supplementary Table 2. A pooled analysis across tectorigenin doses yielded the same conclusion. These results indicate that differences in relaxation are not attributable to differences in pre-contraction. As shown in Fig. 2A,

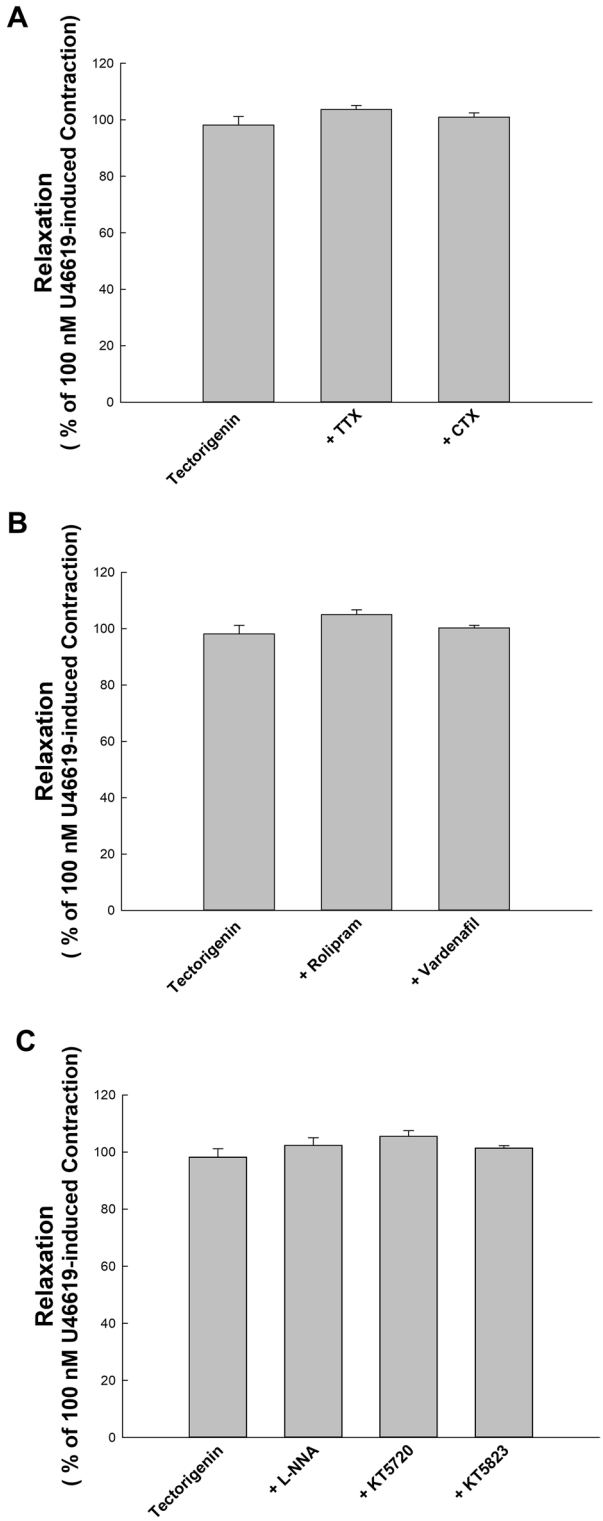

Fig. 2. Effects of neural and cyclic nucleotide pathway inhibitors on tectorigenin-induced relaxation in porcine coronary arteries pre-contracted with 100 nM U46619. (**A**) Tetrodotoxin (TTX, 1 μM) and ω-conotoxin GVIA (CTX, 1 μM) had no significant effect on the relaxation induced by 30 μM tectorigenin ($p > 0.05$, $n = 4$). (**B**) Pretreatment with rolipram (1 μM, a selective phosphodiesterase-4 inhibitor) or vardenafil (1 μM, a selective phosphodiesterase-5 inhibitor) did not significantly alter tectorigenin-induced vasorelaxation ($p > 0.05$, $n = 4$). (**C**) Inhibitors of the nitric oxide and cyclic nucleotide pathways, including Nω-nitro-L-arginine (L-NNA, 100 μM), KT5720 (1 μM, a PKA inhibitor), and KT5823 (1 μM, a PKG inhibitor), also did not significantly affect the relaxant response to tectorigenin ($p > 0.05$, $n = 4$). Data are expressed as mean ± standard error of the mean (SEM) from four independent hearts. U46619 plateau (normalised to 60 mM KCl) was similar across groups ($p > 0.05$; Supplementary Table 2).

the relaxation effect of 30 μM tectorigenin on porcine coronary arteries was not significantly affected by the presence of 1 μM tetrodotoxin (TTX) or 1 μM ω-conotoxin GVIA (CTX) ($p > 0.05$, both $n = 4$).

Impact of rolipram and vardenafil on Tectorigenin-Induced relaxation

Figure 2B depicts that the addition of 1 μM rolipram or 1 μM vardenafil did not enhance the vasorelaxant effect of 30 μM tectorigenin on porcine coronary arterial rings ($p > 0.05$, both $n = 4$).

Role of Cyclic adenosine monophosphate (cAMP), Cyclic Guanosine monophosphate (cGMP) and NO in Tectorigenin-Induced relaxation

As presented in Fig. 2C, the vasorelaxant response induced by 30 μM tectorigenin in porcine coronary arteries was not significantly reduced by 100 μM Nω-nitro-L-arginine (L-NNA), 1 μM KT5823, or 1 μM KT5720 ($p > 0.05$, all $n = 4$).

Influence of potassium channels on Tectorigenin-Induced relaxation in Porcine coronary arteries

As shown in Fig. 3A, pre-treatment with potassium channel inhibitors [100 nM apamin (small-conductance calcium-activated potassium [SKCa] channel blocker), 200 nM iberiotoxin (IbTX, large-conductance calcium-activated potassium [BKCa] channel blocker), 1 mM tetraethylammonium (TEA, non-selective potassium channel blocker), 1 μM charybdotoxin (mixed BKCa and voltage-gated potassium [Kv] channel inhibitor), and 10 μM glibenclamide (ATP-sensitive potassium [KATP] channel blocker)] did not significantly alter the vasorelaxant response to 30 μM tectorigenin ($p > 0.05$, $n = 4$ each). However, 1 mM 4-aminopyridine (4-AP, voltage-gated potassium [Kv] channel inhibitor) significantly reduced the relaxation response ($p < 0.05$, $n = 4$). Further analysis (Fig. 3B) revealed that 1 mM 4-AP significantly inhibited relaxation induced by 10 and 30 μM tectorigenin ($p < 0.05$, $n = 4$), but had no significant effect at 100 μM ($p > 0.05$, $n = 4$). Additionally, Fig. 3C shows that tectorigenin induced significant relaxation in coronary arteries pre-contracted with U46619, whereas no relaxation was observed in those pre-contracted with 80 mM potassium chloride (KCl) ($p < 0.05$, $n = 4$).

Influence of ERs on Tectorigenin-Induced relaxation in Porcine coronary arteries

As shown in Fig. 4A, pre-treatment with 10 μM MPP significantly reduced relaxation induced by 10 and 30 μM tectorigenin ($p < 0.05$, $n = 4$). Similarly, 10 μM PHTPP significantly reduced relaxation at both 10 and 30 μM tectorigenin ($p < 0.05$, $n = 4$). However, neither antagonist significantly affected the relaxation response to 100 μM tectorigenin ($p > 0.05$, $n = 4$).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis (Fig. 4B) showed that ESR1 expression was significantly higher than ESR2, with an approximately six-fold difference ($n = 3$ hearts).

Immunohistochemical analysis of ERα and ERβ expression in coronary arteries

Figure 5A illustrates strong ERα immunoreactivity in the medial smooth muscle (arrows) ($n = 3$ hearts), whereas control sections (Fig. 5B) exhibited no specific staining. In contrast, ERβ expression was markedly weaker (Fig. 5C, $n = 3$ hearts), with corresponding negative controls showing no detectable staining (Fig. 5D). Because all functional assays were performed in endothelium-denuded rings, endothelial ER staining does not contribute to the measured relaxant responses in this study.

Discussion

This study provides novel insights into the cardiovascular actions of tectorigenin, an isoflavone with emerging therapeutic potential. We demonstrated that tectorigenin induces robust, concentration-dependent vasorelaxation in U46619-pre-contracted porcine coronary arteries, supporting its candidacy as a vasoprotective agent for IHD.

Although several isoflavones, including genistein, daidzein, and puerarin, are known to enhance endothelial NO synthesis and modulate calcium or potassium channels in vascular smooth muscle[13–16], our findings suggest a distinct mechanism of action for tectorigenin. The vasorelaxant effect of tectorigenin persisted despite inhibition of neuronal activity (TTX, CTX), cyclic nucleotide pathways (rolipram, vardenafil), protein kinases (KT5720, KT5823), and nitric oxide synthase (L-NNA), indicating that classical endothelium-dependent vasodilatory mechanisms are not primarily involved.

A major mechanistic finding is the involvement of 4-AP-sensitive Kv channels. Tectorigenin-induced relaxation was significantly attenuated by 4-AP but not by other potassium channel blockers (TEA, iberiotoxin, glibenclamide), suggesting a selective activation of Kv channels. Furthermore, the loss of relaxation under high-K^+ (80 mM) depolarisation conditions—where Kv-mediated hyperpolarisation is suppressed—further reinforces the essential role of Kv channel activation. Unlike genistein, which modulates both BKCa and Kv channels[17,18], tectorigenin appears to preferentially target Kv channels, as evidenced by its 4-AP sensitivity and depolarisation-dependent inhibition.

Our data also support the involvement of oestrogen receptors in the vasorelaxant effects of tectorigenin. Both ERα and ERβ antagonists (MPP and PHTPP) attenuated the response, although molecular and immunohistochemical analyses revealed predominant ERα expression in porcine coronary arteries. These findings are consistent with ERα predominance, with ERβ contribution via complementary pathways (e.g., modulation of ionic conductance or vascular smooth-muscle phenotype)[19].

At concentrations near the EC_{50} (10–30 μM), relaxation was attenuated by ER antagonists (MPP, ERα; PHTPP, ERβ) or by 4-AP, implicating ER signalling and 4-AP-sensitive Kv channels. Because combined blockade (ER antagonist + 4-AP) was not tested, additivity vs. occlusion cannot be determined, nor can we distinguish a serial ER→Kv arrangement from a parallel/convergent model; a direct ER-Kv coupling was not established.

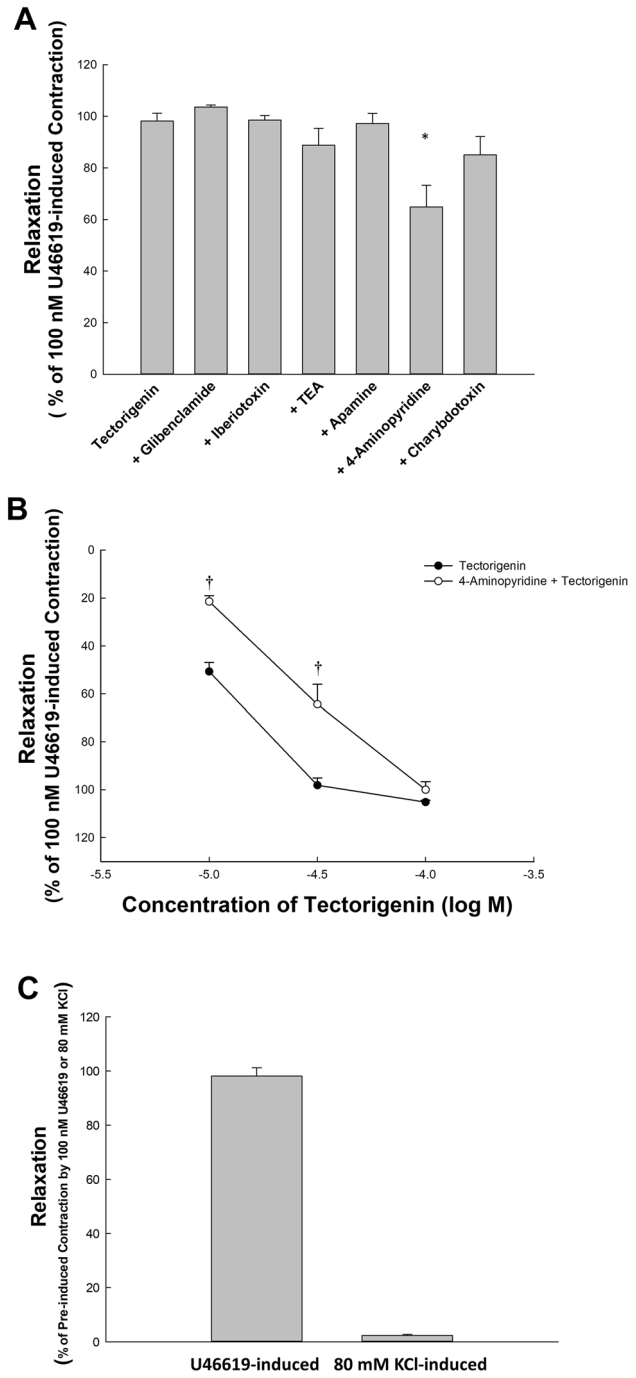

Fig. 3. Involvement of potassium channels in tectorigenin-induced vasorelaxation in porcine coronary arteries. (**A**) Effects of various potassium channel blockers on vasorelaxation induced by 30 μM tectorigenin. Pretreatment with glibenclamide (10 μM), iberiotoxin (IbTX, 200 nM), tetraethylammonium (TEA, 1 mM), apamin (100 nM), or charybdotoxin (1 μM) did not significantly affect the relaxant response ($p > 0.05$), whereas 4-aminopyridine (4-AP, 1 mM) significantly attenuated tectorigenin-induced relaxation ($p < 0.05$). (**B**) Concentration-dependent inhibitory effect of 4-AP (1 mM) on tectorigenin-induced relaxation. Significant inhibition was observed at 10 and 30 μM (†$p < 0.05$ vs. corresponding tectorigenin alone), but not at 100 μM. (**C**) Comparison of the relaxant effects of 30 μM tectorigenin in porcine coronary arteries pre-contracted with either 100 nM U46619 or 80 mM KCl. Tectorigenin elicited significant vasorelaxation in U46619-pre-contracted rings but had negligible effect in KCl-contracted rings. Data are expressed as mean ± standard error of the mean (SEM) from four independent hearts. U46619 plateau (normalised to 60 mM KCl) was similar across groups ($p > 0.05$; Supplementary Table 2).

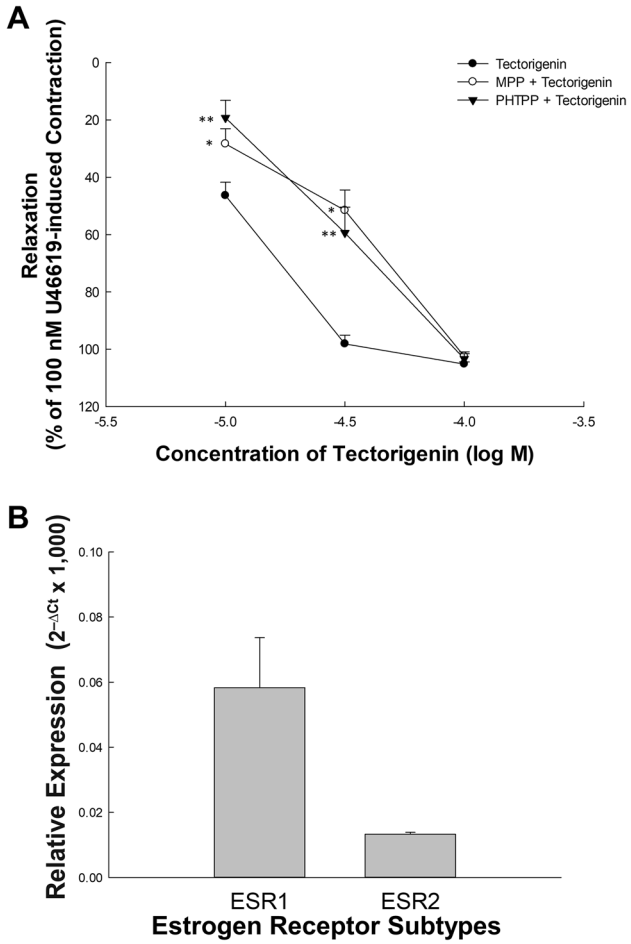

Fig. 4. Involvement of oestrogen receptors in tectorigenin-induced vasorelaxation in porcine coronary arteries. (**A**) Concentration-dependent effects of oestrogen receptor antagonists on tectorigenin-induced relaxation. Pretreatment with methyl-piperidino-pyrazole (MPP) (10 μM, ERα antagonist) or PHTPP (10 μM, ERβ antagonist) significantly attenuated relaxation induced by 10 μM and 30 μM tectorigenin, but not by 100 μM ($p < 0.05$). Statistical significance is indicated by * $p < 0.05$ for MPP + tectorigenin versus tectorigenin alone, and ** $p < 0.05$ for PHTPP + tectorigenin versus tectorigenin alone. (**B**) RT-qPCR analysis of oestrogen receptor subtype expression in porcine coronary arteries. Expression of ESR1 (ERα) was significantly higher than that of ESR2 (ERβ), with approximately a six-fold difference. Data are presented as mean ± standard error of the mean (SEM) from four independent hearts in (**A**) and three in (**B**). U46619 plateau (normalised to 60 mM KCl) was similar across groups ($p > 0.05$; Supplementary Table 2).

Prior reports indicate that ER signalling can stimulate Kv activity in vascular smooth muscle[19,20], but our single-concentration antagonist design was not intended to infer antagonist mechanism (e.g., competitive) or to rank ERα vs. ERβ.

Interestingly, at higher concentrations (100 μM), the inhibitory effects of both ER antagonists and 4-AP were diminished. This observation may reflect receptor saturation, reduced antagonist efficacy, or involvement of additional mechanisms beyond ERα–Kv signalling. Although speculative, previous reports indicate that tectorigenin may act as a competitive thromboxane A_2 (TXA_2) receptor antagonist in human platelets[21]. Given that U46619 is a TXA_2 receptor agonist, this raises the possibility that partial antagonism could influence vascular tone at high concentrations. However, this potential contribution remains to be confirmed, and future studies using alternative contractile agents may help clarify whether TXA_2 receptor interactions play a role in the vasorelaxant profile of tectorigenin.

These mechanistic findings reinforce the broader regulatory roles of oestrogen receptors in vascular homeostasis. ERα and ERβ modulate vascular tone, oxidative stress, and fibrotic remodelling, and are considered protective in cardiovascular disease[22]. Our data support the concept that plant-derived isoflavones such as tectorigenin may mimic endogenous oestrogenic signalling in the vasculature.

While endothelial denudation may reduce physiological relevance, it enabled focused interrogation of smooth muscle-specific mechanisms. This is particularly pertinent in IHD, where endothelial dysfunction is prevalent. Although non-endothelial nitric oxide synthase isoforms (e.g., nNOS or iNOS) may exist in vascular smooth muscle[23], the lack of effect by L-NNA suggests that NO does not contribute significantly to tectorigenin-induced vasorelaxation under these conditions.

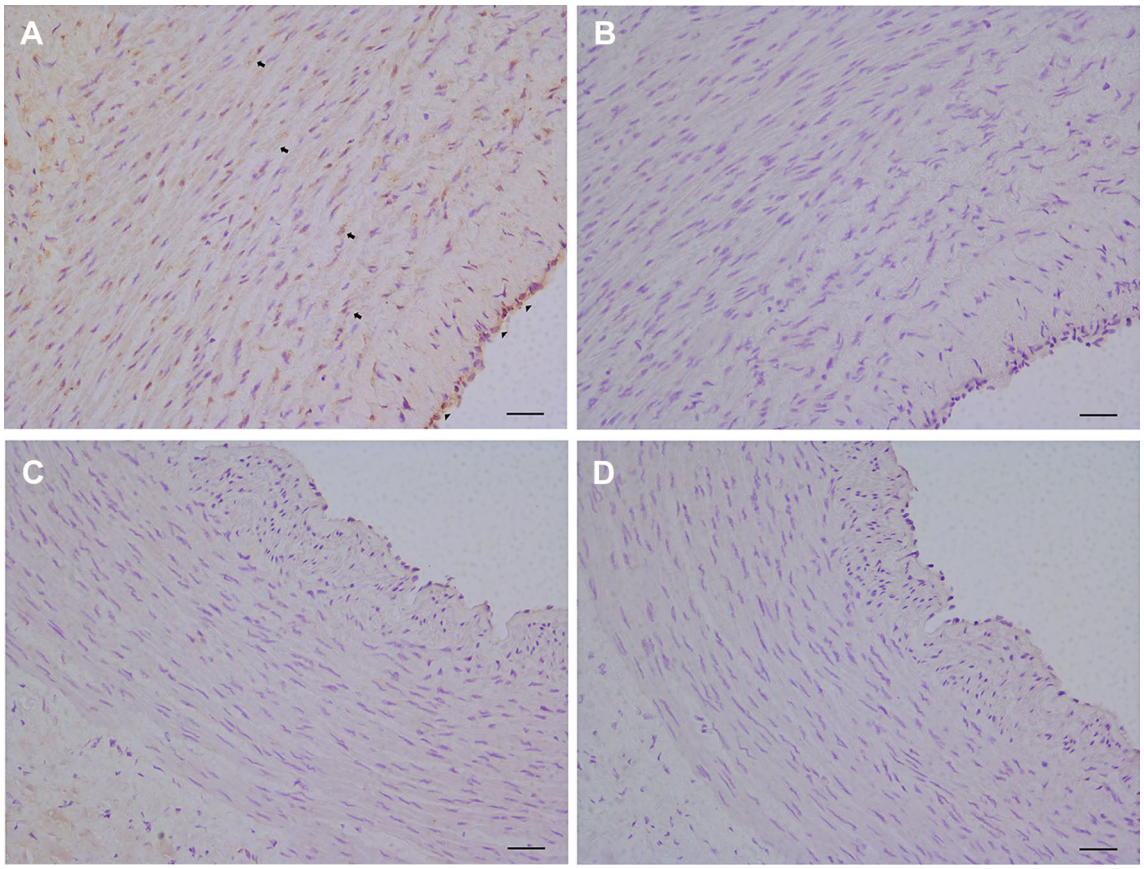

Fig. 5. Immunohistochemical analysis of oestrogen receptor expression in porcine coronary arteries. (**A**) Strong immunoreactivity for ERα (brown staining) was observed in the medial smooth muscle (arrows); the luminal endothelium is indicated by arrowheads. (**B**) Negative control for ERα, showing no specific staining in the absence of the primary antibody. (**C**) ERβ immunoreactivity was markedly weaker in coronary artery smooth muscle cells. (**D**) Negative control for ERβ, showing no detectable staining in the absence of the primary antibody. Sections for immunohistochemistry were prepared without mechanical denudation to preserve endothelium. Images are representative of $n = 3$ hearts. Scale bar = 20 µm.

Several limitations should be acknowledged. Although porcine coronary arteries are widely regarded as a physiologically relevant model for human vessels, confirmatory studies using human vascular tissues are necessary to validate these results. Moreover, this study was conducted under acute ex vivo conditions, and thus does not account for systemic pharmacokinetics, metabolic processing, or off-target interactions. Tissues were obtained from market-weight pigs with sex not recorded, and experiments were not stratified by age or sex; potential age- or sex-related differences in response magnitude therefore cannot be excluded. Because functional assays used endothelium-denuded rings whereas IHC used intact segments, intact-vessel responses may include additional endothelium-dependent components (e.g., NO) that were not assessed here. Western blotting and loss-of-function assays (e.g., siRNA) were not performed; accordingly, attribution to specific ER subtypes and Kv isoforms remains provisional. Future work will address this with Western blot confirmation, targeted knockdown/knockout, and selective Kv-subfamily pharmacology. Expression and localisation datasets (IHC and RT-qPCR) were limited ($n = 3$) and should be interpreted as supportive rather than definitive. In vivo investigations are warranted to evaluate the bioavailability, efficacy, and safety of tectorigenin under physiological conditions. Notably, its low oral bioavailability and susceptibility to metabolism by gut microbiota[7,24] may influence its pharmacological profile and therapeutic viability. These considerations highlight the need for further translational studies before clinical application can be pursued.

Taken together, our findings highlight that tectorigenin induces significant, concentration-dependent vasorelaxation in porcine coronary arteries, with contributions from oestrogen receptors (predominantly ERα) and 4-AP-sensitive Kv channels. We did not assess combined ER antagonism with 4-AP, nor did we identify Kv subtypes; accordingly, it remains to be established whether the inhibitory effects are additive or occlusive, and whether a direct ER-Kv coupling exists. Because antagonists were tested at a single concentration without Schild analysis, the relative contributions of ERα versus ERβ cannot be determined. These mechanisms are distinct from those of other well-studied isoflavones, underscoring a unique pharmacological profile. Our results support the potential of tectorigenin as a vasoprotective agent and warrant further in vivo studies to assess its pharmacokinetic properties, safety, and translational relevance in ischaemic cardiovascular disease.

Methods

Materials acquisition and preservation

Porcine hearts were collected from pigs weighing approximately 110 kg. Porcine hearts were obtained post-mortem from a government-approved abattoir in Taiwan following routine stunning and exsanguination for food production; no live animals were handled or euthanised by the investigators. Information on sex was unavailable at procurement; tissues were not stratified by age or sex. The hearts were immediately submerged in chilled Krebs–Henseleit solution (composed of 118 mM NaCl, 4.7 mM KCl, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$, 1.8 mM $CaCl_2$, and 14 mM glucose; pH adjusted to 7.4) to preserve their physiological integrity. Before use, the Krebs–Henseleit solution was oxygenated with 95% O_2 and 5% CO_2 for 15 min to maintain optimal tissue viability. Next, the tissues were quickly transported to the research laboratory within approximately 30 min.

Because pig hearts are classified as food products rather than live animal subjects, this study was exempted from review by the Institutional Animal Care and Use Committee at E-Da Hospital, in accordance with relevant regulations. However, all procedures strictly adhered to the ethical standards for the handling and use of animal tissues in research.

For experimental assays, a range of pharmacological agents was utilised, including U46619, apamin, KT5720, KT5823, and L-NNA (Sigma-Aldrich, MO, USA); rolipram, vardenafil, and TEA (Santa Cruz Biotechnology, CA, USA); IbTX (Alomone Labs, Jerusalem, Israel); glibenclamide (Research Biochemicals International, MA, USA); TTX and 4-AP (Tocris Bioscience, Bristol, UK); CTX (Bachem, Bubendorf, Switzerland); and charybdotoxin, methyl-piperidino-pyrazole (MPP), and PHTPP (Cayman Chemical, MI, USA).

Preparation of tectorigenin

Tectorigenin (≥ 98% purity) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Due to its poor aqueous solubility, a stock solution (100 mM) was first prepared by dissolving tectorigenin in 100% DMSO. Working solutions were freshly diluted in Krebs–Henseleit buffer to the desired final concentrations (1–300 μM) immediately before use. The final DMSO concentration in the organ bath did not exceed 3.0% (v/v). Vehicle control groups with matching DMSO concentrations were included in all experiments.

Assessment of tectorigenin's effects on U46619-Induced Pre-contraction in Porcine coronary arteries

The porcine model serves as a reliable platform for studying human coronary vascular functions due to the anatomical similarities between porcine and human coronary arteries[25].

Following procurement, the epicardial layer was carefully dissected to expose the left anterior descending (LAD) artery. The artery was then sectioned into rings (approximately 1 cm in length, 0.3 cm in diameter). For all functional pharmacology experiments (mechanistic studies), the endothelium was mechanically removed by gently rubbing the luminal surface (as described previously[26] to eliminate confounding indirect effects of endothelium-derived vasoactive factors and thereby isolate the direct effects of tectorigenin on vascular smooth muscle contraction and relaxation.

Next, arterial rings were transferred to a 7 mL organ bath containing 5 mL of Krebs–Henseleit buffer, maintained at 37 °C and continuously aerated with 95% O_2 and 5% CO_2. Each arterial segment was mounted between two surgical silk threads, with one end connected to an isometric force transducer (FORT10g; Grass Technologies, RI, USA). The transducer signals were amplified (Gould Instrument Systems, OH, USA) and recorded using a computer system (BIOPAC Systems, CA, USA). A resting tension of 1.0 g was applied to the rings. After an initial equilibration, rings were challenged with 60 mM KCl in Krebs–Henseleit buffer to verify contractile viability; preparations that failed to develop a stable contraction were excluded.

After three washes with fresh buffer and a 30-min equilibration, rings were pre-contracted with U46619 (100 nM). A stable plateau was typically reached within 10–15 min and maintained for ≥ 30 min; rings were included only if the U46619 plateau varied by ≤ ± 10% during the analysis window. This fixed concentration was selected based on previous studies in porcine coronary arteries demonstrating that submicromolar levels (e.g., 15–100 nM) of U46619 elicit stable and reproducible contractions suitable for evaluating vasorelaxation[27]. In addition, our pilot experiments confirmed that 100 nM U46619 reliably produced a sustained contractile plateau throughout the assay period, enabling consistent assessment of relaxation responses. In our preparation, 100 nM U46619 generated a stable submaximal contraction of ~ 80–90% of the 60 mM KCl tone in the same ring. Relaxation (%) was calculated as the percentage decrease from the contemporaneous U46619 (100 nM)–induced plateau tension in the same ring (within-ring normalisation).

Assessment of tectorigenin's vasorelaxation effects

We assessed the vasorelaxant effects of tectorigenin at concentrations of 10 μM, 30 μM, 100 μM, and 300 μM on porcine coronary artery rings following pre-contraction with 100 nM U46619. Each concentration was applied to a separate ring in non-cumulative experiments. Relaxation responses were expressed as a percentage of the U46619-induced contraction. A concentration–response curve was generated, and the EC_{50} value was calculated using nonlinear regression.

Influence of neural conduction on Tectorigenin-Induced relaxation

To investigate the role of neural conduction in tectorigenin-mediated vasorelaxation, the organ bath was pre-incubated with either 1 μM TTX, a selective neuronal sodium channel inhibitor, or 1 μM CTX, a neuronal calcium channel blocker, followed by U46619-induced pre-contraction. After a 15-min incubation, 30 μM tectorigenin was added[28]. By comparing the extent of tectorigenin-induced relaxation after inhibitor treatment, the experiment evaluated whether pre-treatment with these inhibitors affects the vasorelaxant effect of tectorigenin.

Impact of rolipram and vardenafil on Tectorigenin-Induced relaxation

We explored whether enhancing cyclic nucleotide signalling pathways could influence tectorigenin-induced vasorelaxation. Rolipram (a selective phosphodiesterase-4 [PDE-4] inhibitor to increase cAMP) and vardenafil (a phosphodiesterase-5 [PDE-5] inhibitor to increase cGMP) were assessed[29]. Coronary artery rings were pre-incubated with 1 μM of either drug for 20 min, followed by U46619-induced pre-contraction. After a 30-min incubation, 30 μM tectorigenin was added.

Role of cAMP, cGMP, and NO in Tectorigenin-Induced relaxation

We examined the involvement of cyclic nucleotides (cAMP and cGMP) and NO in tectorigenin's vasorelaxant effects using specific inhibitors including 1 μM KT5720 (a cAMP-dependent protein kinase [PKA] inhibitor), 1 μM KT5823 (a cGMP-dependent protein kinase [PKG] inhibitor), and 100 μM L-NNA (a NO synthase inhibitor)[28]. The coronary artery rings were pre-incubated with these inhibitors, followed by U46619-induced pre-contraction. After a 30-min incubation, 30 μM tectorigenin was added.

Role of potassium channels and High-Potassium-Induced depolarisation

We investigated the role of potassium channels using specific inhibitors: 1 mM TEA (non-selective potassium channel blocker), 100 nM apamin (small-conductance SKCa), 200 nM IbTX (BKCa), 1 mM 4-AP (Kv), 1 μM charybdotoxin (BKCa and Kv), and 10 μM glibenclamide (KATP)[28–31]. Rings were pre-treated, pre-contracted with U46619, then exposed to 30 μM tectorigenin. High-K^+ (80 mM KCl) Krebs–Henseleit was used to induce membrane depolarisation, to confirm potassium channel involvement by testing tectorigenin under these conditions.

Exploration of ER in Tectorigenin-Induced vasorelaxation

We evaluated the role of ERs by pre-incubating with either 10 μM MPP (ERα antagonist)[32] or 10 μM PHTPP (ERβ antagonist)[33] for 30 min, followed by U46619-induced pre-contraction, and then adding tectorigenin at 10, 30, or 100 μM.

Immunohistochemical analysis of ERs

Segments of LAD arteries were fixed, embedded, sectioned, retrieved, and processed for immunohistochemistry using primary antibodies against ERα and ERβ, visualised with HRP-conjugated secondary antibody and DAB, counterstained with haematoxylin. Controls omitted primary antibodies. For immunohistochemistry only, separate LAD segments were processed without prior mechanical denudation to preserve the endothelial layer for localisation. For IHC, one arterial segment per heart was processed and analysed ($n = 3$ hearts).

RNA isolation and qPCR

Total RNA was extracted (RNA Isolater, Vazyme), reverse-transcribed (HiScript III, Vazyme), and qPCR performed on ESR1 and ESR2 normalised to $\sqrt{(GAPDH \times ACTB)}$ using the $2^{-\Delta Ct}$ method[34]. Primer sequences are in Supplementary Table 1. For RT-qPCR, one LAD sample per heart was analysed ($n = 3$ hearts).

Statistical analysis

For functional assays, one ring per heart per condition was analysed; therefore n (rings) = number of hearts for each condition. For molecular assays (RT-qPCR, IHC), one sample per heart was used ($n = 3$ hearts). Between-group differences in normalised U46619 plateau tension were assessed by one-way ANOVA with Dunnett's multiple comparisons versus the no-inhibitor control ($\alpha = 0.05$; adjusted p values reported). For other comparisons, we used Student's t-test or one-way ANOVA with Tukey's post hoc test, as appropriate. Data are mean ± SEM; $p < 0.05$ was considered significant. SigmaPlot 12.0 (Systat Software, USA) was used for curve fitting and EC_{50} calculations.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

T.-Y.C. and C.-C.T. designed the study. T.-Y.C. conducted investigation, wrote the original draft, and acquired funding. S.-N.Y. contributed to conceptualisation and overall supervision; refined the methodology and the statistical analysis plan; interpreted the data; and substantially revised the manuscript in response to reviewers. H.-P.K. contributed to investigation, visualisation, review and editing, and funding acquisition. W.-H.K. contributed to investigation, visualisation, data curation, and drafting of the original manuscript. I.-C.L. performed data curation and provided resources. L.-W.L. contributed methodology and resources. T.-I.L. contributed data curation, methodology, and resources. L.-C.C., M.-S.W., and H.-C.C. provided resources. C.-C.T. supervised conceptualisation, methodology, and validation, acquired funding, and critically reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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