Vasorelaxant Effects of Ethyl Cinnamate Isolated from *Kaempferia galanga* on Smooth Muscles of the Rat Aorta

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**Abstract**

From the rhizomes of *Kaempferia galanga*, ethyl cinnamate (EC) was isolated and its vasorelaxant effect was examined on the rat aorta. EC inhibited the tonic contractions induced by high K⁺ and phenylephrine (PE) in a concentration-dependent manner, with respective IC₅₀ values of 0.30 ± 0.05 mM and 0.38 ± 0.04 mM. The relaxant effect against PE-induced contractions was greater in the presence of endothelium. Pre-treatment of the aorta with methylene blue and indomethacin significantly reduced the relaxant effect. These results suggest that the inhibitory effects of EC may involve inhibition of Ca²⁺ influx into vascular cells and release of nitric oxide (NO) and prostacyclin from the endothelial cells. Thus, the vasorelaxant effect of EC mediated through multiple pathways may explain the traditional use of the parent plant in treating hypertension.

*Kaempferia galanga* L. (Zingiberaceae) grows wild or is cultivated in India, China, South-east Asia, particularly Malaysia, Indonesia and Singapore [1]. It is widely used as flavouring in food and as an important element in the preparation of "jamu" (a local health tonic). It is also known to treat ailments such as hypertension, rheumatism and asthma. The rhizomes of this plant are used to treat abdominal pain, boiled with other roots for treating women after childbirth, also to treat swelling and muscular rheumatism [2], [3]. The results of our previous study [4] showed that the smooth muscle relaxant activity of the crude extract was mainly due to the inhibition of Ca²⁺ influx through the voltage- and receptor-operated channels, and Ca²⁺ sensitivity of contractile elements. In the present study, EC (1) was isolated, purified from

![Ethyl cinnamate](image-url)

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the rhizome of the plant and identified as the major compound contributing to the vasorelaxant activity.

In rat aorta, high K⁺ (80 mM) caused a tonic contraction while PE (0.1 μM) caused an initial phasic contraction followed by a tonic contraction [5]. The cumulative applications of EC inhibited the sustained contractions induced by high K⁺ and PE with IC₅₀ values of 0.30 ± 0.05 mM and 0.38 ± 0.04 mM, respectively (Fig. 1). Contractions induced by high K⁺ (80 mM) are due to membrane depolarization, which activates L-type voltage dependent channels (VDC) and thus permits Ca²⁺ entry. In addition to VDC, receptor agonists, such as PE, activate receptor-operated Ca²⁺ channels (ROC) to induce the sustained contraction [S]. In a preliminary experiment, we showed that verapamil at 10 μM, markedly reduced both high K⁺ (95 ± 2%; n = 5) and PE (82 ± 5%; n = 5)-induced contractions of the rat aorta. The present findings suggest that EC shares a similar relaxant action to verapamil, a calcium channel blocker [7].

It had been shown that most of the Ca²⁺ channel blockers have additional intracellular sites of action [8]. The relaxant action of EC against PE-induced contractions was compared between endothelium intact and denuded preparations. Following the removal of the endothelium, inhibitory action of the compound against the contractions of the aorta was markedly attenuated (Fig. 2) suggesting the involvement of NO and prostacyclin. NO activates soluble guanylate cycloxe of vascular smooth muscle, and the resulting increase in cyclic GMP levels produces relaxation [9]. Methylene blue is an inhibitor of guanylate cyclase, while indomethacin abolishes the generation of prostaclins by inhibiting the enzyme cyclo-oxygenase, which is involved in the metabolism of arachidonic acid. In this experiment, the rat aorta was pre-treated with methylene blue (10 μM) or indomethacin (20 μM) for 20 minutes before contracting the muscle with 0.1 μM phenylephrine. The relaxant effect of ethyl cinnamate was strongly inhibited by pre-treatment with methylene blue (36%) and indomethacin (71%), thus confirming the additional involvement of NO and prostacyclin in mediating the vasorelaxant action of the compound (Fig. 3). These findings suggested that EC acts upon various sites causing the relaxation of vascular smooth muscles and presence of this compound in Kaempferia galanga may explain the traditional use of the plant in treating hypertension. Interestingly, EC is contained in all red wines as flavour, which might be responsible for the known vasorelaxant effect of red wine [10].

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**Materials and Methods**

Silica gel 60 F₂⁵⁴ (230–400 Mesh ASTM) was used in thin layer chromatography (TLC). Kieselgel (70–230 Mesh ASTM) was used in column chromatography (CC). UV light (254 and 365 nm) was used to examine TLC spots or bands. I₂ vapour was used as staining reagent. Spectral data were obtained as follows: UV on a Shimadzu UV-160A, IR on a Perkin-Elmer 1600 series double-beam recording spectrometer, NMR on a JEOL JNM-LA400 FT NMR system and MS on a Shimadzu GC-MS (GC-17A, MSDP-1600).

_K. galanga_ was obtained from the botanical garden of the University of Malaya and was identified by a botanist, Halijah Ibrahim. The dried rhizomes (5 kg) of this plant were extracted using Soxhlet with petroleum ether and dichloromethane (CH₂Cl₂), consecutively. The dried crude CH₂Cl₂ extract (CEKCL) represented a yield of 2.4% of the dried powder. CEKCL was mixed with petroleum ether, the precipitate was filtered and the mother li-
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CHEMICAL COMPOSITION OF COMPONENTS OF VI-28

Radix Ginseng

- 2-3% Ginsenosides (triterpene saponins)
- 0.05% essential oil (limonene, terpineol, citrol, polyacetylenes)
- sugar
- starch

Corne Cervi Pantotrichum

- 34% Ash
- 12% moisture
- nitrogen
- fats
- collagen
- glycosaminoglycans (chondroitin sulfate, keratin sulfate, hyaluronic acid, dermatan sulfate, chondroitin sulfate proteoglycan, decorin)
- lipids (polysaccharides)
- growth hormone and prostaglandins (IGF-1, IGF-2)

Semen Cuscutae

- quercetin 3-O-beta-D-galactoside-7-O-beta-D-glucoside (I)
- quercetin 3-O-beta-D-apiofuranosyl-(1-->2)-beta-D-galactoside (II)
- hyperoside (III)
- isorhamnetin (IV)
- kaempferol (V)
- quercetin (VI)
- d-sesamin (VII)
- 9(R)-hydroxy-d-sesamin (VIII)
- Vitamin A

Fructus Cnidii

- osthol
- imperatorin
- xanthotoxin
- isopimpinellin
- bergapten
Kaempferiae Rhizoma
- cineol
- borneol
- 3-carene
- camphene
- kaempferal
- kaempferide
- cinnamaldehyde
- p-methoxycinnamic acid
- ethyl cinnamate
- ethyl p-methoxycinnamate

Inactive Ingredient
- rice powder

Capsule
- Gelatin Capsule

METHOD OF MANUFACTURE

Phase A
Radix ginseng; Cornu Cervi pantotrichum and Rhizome kaempseriae are ground to a fine powder under low temperature.

Phase B
Fructus cnidii and Semen cuscutae are water decocted, filtered, the solution is oven dried, and reduced to a fine powder via grinding process.

Phase A and B are mixed in proper proportion to form the final mix. The final mix is heat treated at 80°C for 24 hours, and then filled into gelatin capsules.

MICROBES AND PESTICIDE CONTROL

In the control of microbes, it is believed the method of manufacture, namely the use of heat treatment 80°C for 24 hours, is sufficient to address any microbes that may be present.

Regarding pesticide monitoring, the final mix is subject to pesticide monitoring in accordance with the Hong Kong Standards and Testing Centre (report of 2003-02-14 enclosed herewith).
TEST ARTICLES UTILIZED IN STUDIES

The FDA has asserted in its reply to the original Notification that, at page 3, second full paragraph, there are discrepancies with the description of the test articles used in studies submitted with the original Notification. Specifically, the FDA states that one study reported was conducted with VI-28, and other studies were conducted with test substances similar to VI-28, however "the relationship of these test materials to the botanical preparations that are the subject of the notification was not stated".

With reference to the letter faxed to Dr. Walker on 21 January 2004 (attached herewith), it was previously stated that VI-28 to be marketed consists of the botanical preparations that were the subject of the original Notification (and subsequently this re-submitted Notification). Because the botanical preparations are not modified chemically in the preparation of VI-28 and the preparations are believed to be safe, it is believed that VI-28 to be marketed is safe. The test materials used in the studies are qualitatively and quantitatively akin to VI-28 to be marketed in the U.S., i.e., it consists of the same botanical preparations used in the same amounts as stated in this Notification.