Biphasic effect of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract on rat isolated vascular smooth muscles

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Abstract

In this study, we examined the effects of *Psidium guajava* Linn. leaf aqueous extract (PGE) on isolated, spontaneously-contracting portal veins, as well as on endothelium-intact and endothelium-denuded descending thoracic aortic ring preparations of healthy, normotensive rats. Graded concentrations of PGE (0.25–4.0 mg/ml) caused concentration-dependent, initial brief but significant (\(P<0.05\)) rises of the basal tones and amplitudes of pendular, rhythmic contractions, followed by secondary pronounced, longer-lasting and significant (\(P<0.05–0.001\)) inhibitions of contractile amplitudes of the isolated portal veins. Relatively low concentrations of PGE (<1.0 mg/ml) always contracted freshly-mounted, naïve, endothelium-intact aortic ring preparations. However, relatively high concentrations of PGE (1.0–4.0 mg/ml) always produced initial brief contractions/augmentations of noradrenaline (NA, \(10^{-7}\)M)-induced contractions of endothelium-intact and endothelium-denuded aortic ring preparations, followed by secondary, pronounced relaxations of the aortic ring muscles. Moreover, relatively high concentrations of PGE (1.0–4.0 mg/kg) always relaxed NA-induced contractions of the aortic ring preparations in a concentration-related manner. The arterial-relaxing effects of PGE were more pronounced in endothelium-intact aortic rings than in endothelium-denuded aortic ring preparations. The relaxant effects of PGE on endothelium-intact aortic rings were only partially inhibited by \(\text{NO}-\text{nitro-L-arginine methyl ester (L-NAME, 100 } \mu\text{M)}\), a nitric oxide synthase inhibitor, suggesting that the vasorelaxant effect of PGE on aortic rings is probably mediated via both endothelium-derived relaxing factor (EDRF)-dependent and EDRF-independent mechanisms. Taken together, the findings of this study indicate that PGE possesses a biphasic effect on rat isolated vascular smooth muscles.

Key words: rat, vascular smooth muscle, *Psidium guajava* leaf aqueous extract, vasorelaxant action, endothelial factors

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Introduction

*Psidium guajava* Linn. (family: Myrtaceae) is an important food crop and medicinal plant (Gutierrez et al., 2008). It grows in all tropical and subtropical parts of the world, and readily adapts to different climatic conditions. Although a native of Central America, *Psidium guajava* has become naturalised in many other parts of the world, including South Africa. *Psidium guajava*, popularly known in English as ‘Guava’ and as ‘ugwava’ in isiZulu, is a shrub or small tree of about 2–5 m in height. The bark peels off in flakes, revealing the characteristically smooth trunk (Van Wyk et al., 2002). The bark is thin, smooth, patchy, copper-coloured, and flakes off showing the greenish layer beneath (Kamath et al., 2008). The short-petiolate, large leaves are formed opposite each other in pairs, with prominent veins, particularly on the lower side. The blade is oval with prominent pinnate veins, 5–12 cm long (Gutierrez et al., 2008). The flowers are somewhat showy; petals whitish, up to 2 cm long and contain numerous stamens. The rounded or pear-shaped yellow fruits are fleshy, globose to ovoid berry, about 5 cm in diameter, with an edible pink mesocarp containing numerous small, hard seeds (Gutierrez et al., 2008). The many-seeded fruits have delicious taste and are high in vitamin C content (Van Wyk et al., 2002).

‘Guava’ leaves are commonly used in South African traditional medicine for a catalogue of human ailments, including fever, cough, ulcers, boils and wounds, malaria and diarrhoea (Van Wyk et al., 2002). Recent ethnopharmacological studies have shown that the leaves of *Psidium guajava* are used traditionally in South African folk medicine to manage, control, and/or treat a plethora of human disorders, including diabetes mellitus and hypertension (Oh et al., 2005; Ojewole, 2005). In other parts of the world, the leaves of *Psidium guajava* are used for many other human ailments, including caries, pyrexia, cough, wounds, bleeding gums, inflammation, epilepsy, kidney problems, gout, pain relief, skin ailments, ulcers, and boils (Conway, 2001; Hutchings et al., 1996; Nadkarni and Nadkarni, 1999; Van Wyk et al., 2002; Watt and Breyer-Brandwijk, 1962). Other reported ethnotherapeutic uses of *Psidium guajava* leaves include gastroenteritis, cholera, dysentery, stomach upsets, bacterial infections, and so on (Gutierrez et al., 2008). Some recent studies have shown that *Psidium guajava* extracts possess antiplasmodial activities on various strains of malarial parasites (Iwu, 1993; Nundkumar and Ojewole, 2002); and contractile effect on rat isolated aortic rings (Olatunji-Bello et al., 2007).

Phytochemical analyses of *Psidium guajava* leaves have revealed the presence of flavonoids, which include quercetin and its derivatives (guajaverin, isoquercitrin, hyperin, quercitrin, avicularin), morin and its derivatives (morin-3-O-α-L-lixopyranoside and morin-3-O-α-L-arabopyranoside), rutin, myricetin, luteolin and kaempferol (Vargas et al., 2006; Gutierrez et al., 2008). The leaves of the plant have also been shown to contain essential oil, fixed oil, volatile oil, saponin, resin, tannin, triterpenoids, asiatic acid and ellagic acid (Arima and Danno, 2002; Begum et al., 2002a, b; Iwu, 1993; Li et al., 1999; Lozoya et al., 1994; Nadkarni and Nadkarni, 1999; Zakaria and Mohd, 1994).

*Psidium guajava* leaves are frequently used in South African traditional medicine for the management of hypertension. In an attempt to provide a pharmacological rationale (or otherwise) for the use of ‘Guava’ leaves in the management of hypertension, the present study
was undertaken to investigate the effects of *Psidium guajava* leaf aqueous extract on mammalian isolated vascular smooth muscles.

**Materials and methods**

**Ethical considerations**

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa; and conform to the “Guide to the Care and Use of Animals in Research and Teaching” (Published by the Ethics Committee of the University of KwaZulu-Natal, Durban 4000, South Africa).

**Plant material**

Fresh leaves of *Psidium guajava* were collected from an open grassland on the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa, in March 2008. Identification of the plant was carried out by the Taxonomist/Curator of the University of KwaZulu-Natal’s Botany Department. A voucher specimen of the plant (CHIWOROROWDH.01) has been deposited in the Herbarium of the University’s Botany Department.

**Preparation of Psidium guajava leaf aqueous extract**

One kilogramme (1 kg) of fresh *Psidium guajava* leaves was air dried under shade at room temperature (26 ± 1°C) for a period of 2 weeks. The dried leaves were thereafter milled into fine powder in a Waring commercial blender. The powdered leaves was macerated in distilled water and extracted twice, on each occasion with 2.5 litres of distilled water at room temperature (26 ± 1°C) for 48 hours (with occasional shaking). The combined aqueous extract solubles were filtered and concentrated under reduced pressure in a rotary evaporator at 60 ± 1°C. Freeze-drying and solvent elimination of the resulting aqueous extract finally gave 36.56 g (i.e., 3.656% yield) of a light brown, powdery crude *Psidium guajava* leaf aqueous extract (PGE). Without any further purification, aliquot portions of PGE were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

**Animals**

Effects of PGE on rat vascular smooth muscles were investigated in isolated, spontaneously-contracting portal veins and descending thoracic aortic ring preparations of naïve normotensive rats. Healthy, young adult, male and female Wistar rats weighing 250–300 g were used. The animals were kept and maintained under conventional laboratory conditions of temperature, humidity and light, and allowed free access to food (standard pellet diet) and drinking tap water *ad libitum*. All the animals used were fasted for 16 hours, but still allowed free access to drinking tap water before the commencement of our experiments. Each rat was euthanased by halothane inhalation, following which the abdomen and thorax were opened, and the portal vein (with an *in situ* length of 2–3 cm), as well as the descending thoracic aorta (4 cm long) were quickly removed from the animal. The harvested muscles were cleaned free from fat and connective tissues, and trimmed.
Effects of PGE on rat isolated portal veins

Each isolated portal vein was suspended under an applied resting tension of 0.5 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution (KHS) of composition, in mM: NaCl, 118; KCl, 4.7; NaHCO₃, 25.0; MgCl₂, 1.2; CaCl₂·2H₂O, 2.52; NaH₂PO₄·2H₂O, 1.28; and glucose, 5.55; pH adjusted to 7.4. The bathing KHS was maintained at 35 ± 1°C and continuously aerated with carbogen (i.e., 95% O₂ + 5% CO₂ gas mixture). The mounted portal vein preparations were subsequently left to equilibrate for 45–60 min, during which time the bathing physiological solution was changed every 15 min, before they were challenged with graded concentrations of PGE (0.25–4.0 mg/ml) or reference drugs used, at different times. PGE and/or reference drug solutions used were added to the bath-fluid either sequentially or cumulatively. Bath-applied PGE and/or reference drug concentrations were repeated (where necessary) after washing out the previous extract or reference drug concentration 4–5 times, and allowing each tissue preparation to rest for 5–10 min, or until its tone returned to the baseline level. In order to make allowance for changes in tissue sensitivity, two isolated portal veins were always set up at a time, one used as ‘control’ and the other one used as ‘test’ (i.e., PGE- or reference drug-treated) preparation. ‘Control’ venous muscle strips were only treated with distilled water equivalent to the volume/s (0.1–0.8 ml) of bath-applied PGE or reference drug solution. To find out whether the contractile effect of PGE on portal vein preparations was mediated through alpha₁-adrenoceptor stimulation or voltage-operated calcium channels, some of the portal vein preparations used were pre-treated with prazosin (10⁻⁶ M), an α₁-adrenoceptor blocker, or nifedipine (10⁻⁶ M), an L-type voltage-operated calcium channel blocker, respectively, 10 min before the addition of PGE to the bath-fluid. PGE- and/or reference drug-induced responses of the isolated venous smooth muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing 2-channel ‘Gemini’ recorders (model 7070).

Effects of PGE on rat isolated aortic ring preparations

Rat isolated descending thoracic aortic ring preparations (3 mm long) suspended in 30-ml Ugo Basile organ-baths containing Krebs-Henseleit physiological solution (maintained at 35 ± 1°C and continuously aerated with carbogen under 1 g resting tensions) were left to equilibrate for 45–60 minutes. During the equilibration period, the bathing KHS was changed every 15 min. After equilibration, the aortic rings were contracted with bath-applied, single submaximal concentrations of noradrenaline (NA, 10⁻⁷ M). When the contractile response to a bath-applied NA concentration was stable, PGE was progressively added to the bath-fluid in geometrically-increasing cumulative concentrations (0.25–4.0 mg/ml) at 2–3 min intervals. In 50% of the isolated thoracic aortic rings used, the endothelium of the aortic ring preparations was mechanically removed (i.e., denuded) in situ by gently rubbing the luminal surfaces of the aortic rings with saline-moistened cotton wool and/or a glass rod. ‘Control’ aortic rings with and/or without functional endothelium (i.e., endothelium-intact and/or endothelium-denuded aortic rings) were pre-contracted with single, submaximal concentrations of NA (10⁻⁷ M). In the endothelium-denuded aortic ring preparations pre-contracted with NA (10⁻⁷ M), the absence or presence of endothelium was confirmed by lack of, or partial (3–5%) relaxant effects to bath-
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applied acetylcholine (ACh, 10⁻⁶ M). In the endothelium-intact aortic rings, the presence of functional endothelium was confirmed by 70–80% relaxation responses to ACh (10⁻⁶ M) of the NA (10⁻⁷ M) pre-contracted preparations. The possible involvement of endothelium-derived relaxing factor (EDRF) in PGE-induced relaxations was investigated in endothelium-intact aortic rings pre-treated with N⁵-nitro-L-arginine methyl ester (L-NAME, 100 µM), a nitric oxide synthase inhibitor. In all cases, PGE- and/or reference drug-induced responses (relaxations or contractions) of the aortic ring preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing 2-channel ‘Gemini’ recorders (model 7070).

Statistical analysis

Experimental data are expressed as means (± SEM). Distilled water-induced ‘control’ means were used as baseline values. The differences in responses among the different groups were analysed for statistical significance using one sample Student’s t-test for paired observations, and two-way analysis of variance (ANOVA, 95% confidence interval – GraphPad PRISM software, Version 5.00) for paired data, followed by Dunnett’s post-hoc test. Multiple comparisons of the means were also performed using Bonferroni’s test. In all cases, values of P≤0.05 were taken to imply statistical significance.

Results

Effects of PGE on rat isolated portal veins

The rat isolated portal veins used always exhibited spontaneous, myogenic, rhythmic contractions. PGE (0.5–4.0 mg/ml) raised the basal tones, and caused concentration-dependent and significant (P<0.05–0.001) reductions in the amplitudes of the myogenic, pendular contractions of the rat isolated portal veins. The inhibitory effects of PGE on contractile amplitudes of the isolated portal veins were usually preceded by initial brief but significant (P<0.05) rises in the basal tones and amplitudes of contractions, followed by secondary, pronounced relaxations. Figure 1 shows a typical trace obtained with, while Fig. 2 summarizes the relaxant effects of PGE. The relaxant effects of PGE were reversed by washing out PGE for 4–5 times, and subsequently allowing the venous tissues to rest for 5–10 min. Pre-incubation of the portal vein preparations with prazosin (10⁻⁶ M) did not modify the initial contractile effect of PGE on the isolated venous muscles, whereas pre-incubation of the tissues with nifedipine (10⁻⁶ M) partially attenuated the initial brief stimulant effects of PGE on the isolated portal veins.

Effects of PGE on rat isolated aortic rings

Exogenous additions of NA (10⁻⁷ M) to the bath-fluid provoked tonic, sustained contractions of both endothelium-intact and endothelium-denuded aortic ring preparations. Removal of the functional endothelium did not significantly affect (P>0.05) contractile responses of endothelium-denuded aortic rings to NA, but relaxant responses of the arterial smooth muscles to ACh (10⁻⁶ M) were almost abolished in the endothelium-denuded aortic ring preparations (84 ± 7% and 4 ± 1%, in endothelium-intact and endothelium-denuded preparations, respectively). PGE (1.0–4.0
mg/ml) produced concentration-related relaxations of the contractile responses induced by NA. Relatively low concentrations of PGE (<1.0 mg/ml) always contracted freshly-mounted, naïve endothelium-intact aortic ring preparations. Furthermore, relatively low concentrations of PGE always produced additional contractions (i.e., augmentations) of NA-contracted endothelium-intact aortic ring preparations. However, relatively high concentrations of PGE (1.0–4.0 mg/ml) always produced initial brief contractions, followed by secondary, pronounced relaxations of the NA-contracted endothelium-intact aortic ring preparations (Fig. 3).

Fig. 1. Effects of *Psidium guajava* leaf aqueous extract (PGE) on a rat isolated portal vein. PGE (2.0 mg/ml) was added to the bath-fluid at the left-hand-side solid, upward-pointing arrow; and washed out 4–5 times at the adjacent, right-hand-side open, downward-pointing arrow.

Fig. 2. Concentration-effect curve of *Psidium guajava* leaf aqueous extract (PGE) on rat isolated portal veins. Values presented are means (± SEM) of 6–8 observations, while vertical bars denote standard errors of the means (SEM). *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001 versus control.
Generally, PGE (1.0–4.0 mg/ml) produced more marked relaxant responses in the endothelium-intact aortic ring preparations than in endothelium-denuded tissues (Fig. 4). The IC₅₀ values for PGE-induced relaxations of NA-provoked contractions of endothelium-intact and
endothelium-denuded aortic rings were $0.69 \pm 0.04$ mg/ml and $1.42 \pm 0.07$ mg/ml, respectively.

The relaxant effects of PGE in endothelium-intact aortic rings were partially (40–55%) and significantly ($P<0.05$), but not completely, inhibited by nitric oxide synthase inhibitor, L-NAME (100 $\mu$M), suggesting that the vasorelaxant effect of PGE was, at least in part, mediated via endothelium-dependent relaxing factor (EDRF) (Fig. 5).

### Discussion

The results of the present study indicate that PGE possesses biphasic effects on rat isolated vascular smooth muscles. However, the PGE-provoked initial brief contractions of the venous smooth muscle preparations were not modified by preincubation of the vascular smooth muscles with prazosin. This observation probably suggests that the initial brief contractile effects of PGE on basal tones and amplitudes of the isolated portal veins are unlikely to be mediated via $\alpha_1$-adrenoceptor stimulation. Relatively low concentrations of PGE (<1 mg/ml) always contracted freshly-mounted, naïve endothelium-mounted, aortic ring preparations. Moreover, high concentrations of the plant’s extract (PGE, 1.0–4.0 mg/ml) always produced initial brief contractions, followed by secondary, pronounced relaxations of NA-contracted, endothelium-intact, aortic ring preparations. In an earlier study, Olatunji-Bello et al. (2007) observed that PGE (0.25–2.0 mg/ml) significantly contracted rat isolated aortic rings in a concentration-related manner. The findings of the present study are, therefore, in agreement with, and extend the work reported by Olatunji-Bello et al. (2007).

Removal of functional endothelium from the aortic rings used did not completely abolish PGE-provoked relaxations of the endothelium-denuded aortic ring preparations pre-contracted
with NA. This finding suggests possible involvement of both EDRF-dependent and EDRF-independent vasodilation mechanisms. EDRF-dependent involvement was corroborated by the partial inhibition of PGE-induced vasorelaxation by L-NAME (Rees et al., 1989; Baisch et al., 2004). The partial blockade of the PGE-elicited initial brief contractions of the rat isolated portal veins by nifedipine probably suggests partial increase of influx of extracellular Ca$^{2+}$ through L-type voltage-sensitive channels (Navasa et al., 1991).

Previous studies in our laboratories and elsewhere have shown that quercetin, kaempferol, morin, myricetin, luteolin and rutin, all present in PGE, possess vasodilator effects in rat isolated aorta and porcine coronary artery (Duarte et al., 1993a, b; Herrera et al., 1996; Pérez-Vizcaino et al., 2002; Ajay et al., 2003; Xu et al., 2007). In the present study, we report for the first time in biomedical literature, that *Psidium guajava* crude leaf aqueous extract, which contains many flavonoids, especially quercetin, induces vascular smooth muscle relaxation, with potency lower than that of pure quercetin (Chiwororo and Ojewole, 2008 — unpublished observation).

The possible role of endothelium mediators such as cyclic nucleotides, in PGE-induced vasodilation was investigated by using endothelium-denuded aortic ring preparations. The vasorelaxant effect of *Psidium guajava* leaf aqueous extract was found to be smaller in endothelium-denuded aortic ring preparations, suggesting that PGE-induced vasodilation might be mediated, at least in part, through EDRF mechanisms. Acute activation of endothelial nitric oxide synthase (eNOS) by ACh results in the synthesis and/or release of endothelial nitric oxide (NO), which in turn leads to guanylate cyclase activation, cyclic guanosine monophosphate (cGMP) elevation, leading ultimately to vascular smooth muscle relaxation (Ajay et al., 2006). Recent available data suggest that the vascular beneficial effects of flavonoids are closely related to their free radical scavenging and antioxidant properties, which might thus protect NO from superoxide-induced inactivation (Rice-Evans and Packer, 1998; Ajay et al., 2006). Quercetin is a potent antioxidant, and has been shown to protect nitric oxide from scavenging actions of superoxide anion (Ajay et al., 2006). In some reports, the vasorelaxation induced by quercetin (and other related flavonoids) was endothelium-independent (Duarte et al., 1993a, Pérez-Vizcaino et al., 2002), or very weakly (less than 2-fold shift) inhibited by endothelial removal (Chen and Pace-Asciak, 1996). However, in a chronic study by Duarte et al. (2001), quercetin restored endothelium-dependent relaxation. Studies by Duarte et al. (2001) and Ajay et al. (2006), suggest that the effects of quercetin are more pronounced when bioavailability of endothelium-derived nitric oxide (EDNO) is impaired. Flavonoids have recently been reported to elicit endothelium-derived hyperpolarizing factor (EDHF)-mediated vascular reactivity in vascular preparations (De Moura et al., 2004; Woodman and Boujaoude, 2004). However, Xu et al., (2007) reported in their study that it is unlikely that flavonoids induce vascular muscle relaxation through hyperpolarization via activation of potassium channels.

An increase in intracellular Ca$^{2+}$ concentration, and the subsequent Ca$^{2+}$-calmodulin-dependent activation of myosin light chain kinase, is the main determinant of smooth muscle contraction (Somlyo and Somlyo, 2000). However, many studies have shown that agonist drugs can also modulate contractile force by increasing myofilament sensitivity to Ca$^{2+}$, or through Ca$^{2+}$-independent pathways (Somlyo and Somlyo, 2000). The involvement of protein kinases, such as protein kinase C or Rho-kinase, in the signaling cascades of Ca$^{2+}$-sensitization in intact and
permeabilized arteries has been reported (Martínez et al., 2000; Somlyo and Somlyo, 2000). Quercetin and kaempferol have also been reported to inhibit Ca\(^{2+}\)-sensitizing mechanisms for smooth muscle contractions (Middleton et al., 2000). In a study by Duarte et al. (1994), quercetin did not modify \(^{45}\text{Ca}^{2+}\) efflux induced by noradrenaline, and at a concentration of \(3 \times 10^{-5}\) M, quercetin only weakly inhibited \(^{45}\text{Ca}^{2+}\) influx induced by KCl in rat aortae. In a study by Pérez-Vizcaíno et al. (2002), the potent vasodilator effects of quercetin and its metabolite, isorhamnetin, in permeabilized iliac arteries at constant intracellular Ca\(^{2+}\) concentrations, confirmed that changes in intracellular Ca\(^{2+}\) concentrations are not required for quercetin’s vasodilator effect. Quercetin’s vasodilator effects, without changes in intracellular Ca\(^{2+}\) concentrations, in the absence of vasoconstrictor agonists or other Ca\(^{2+}\)-sensitizing agents, strongly suggest that they are related to direct interactions with contractile proteins (Pérez-Vizcaíno et al., 2002). In fact, flavonoids, including quercetin and kaempferol, are potent inhibitors of myosin light chain kinase in vascular smooth muscles, with IC\(_{50}\) values of 1 and 0.45 \(\mu\)M, respectively (Hagiwara et al., 1988; Rogers and Williams, 1989), which corresponded to the range of concentrations that exhibited vasodilator effects in the study by Pérez-Vizcaíno et al. (2002).

Protein kinase C (PKC) has been proposed to play a key role in the maintenance of tonic contractions of vascular smooth muscles (Rasmussen et al., 1987). PKC from a rat brain was inhibited by plant flavonoids in a concentration-dependent manner, depending on the flavonoid’s structure (Ferriola et al., 1989). In studies by Duarte et al. (1993a, b), the vasoconstriction induced by protein kinase C activator, phorbol 12-myristate-13-acetate (PMA), was inhibited by quercetin and related flavonoids in the rat aorta. From these studies and our results, it is not unreasonable to speculate that the primary mechanism of PGE- or flavonoid-induced vasodilation results from inhibition of protein kinases, such as myosin light chain kinase, and possibly, other kinases involved in Ca\(^{2+}\)-sensitizing mechanisms, including protein kinase C.

Considering their structural similarities, it is reasonable to postulate common signaling mechanisms for the way in which flavonoids exert their vascular actions (Xu et al., 2007). However, several investigators have shown that the effects of different flavonoids on smooth muscles vary, depending on their structure-activity relationships (SAR) (Hiipakka et al., 2002; Taubert et al., 2002). In a comparative study, Duarte et al. (1993b) found that the order of potency of some flavonoids in relaxing vascular smooth muscle contractions induced by different agents was: flavonols > flavones > flavanols. According to Xu et al. (2007), flavanols (i.e., quercetin, morin and myricetin) have reduced relaxant activities compared to flavones (luteolin). Kaempferol, a member of the flavonols, possesses less –OH groups in its B ring, and thus has more pronounced relaxant effect, compared to its counterparts.

The relaxant effects of PGE on portal vein strips were usually preceded by initial brief but significant rises (\(P<0.05\)) in the basal tones and amplitudes of contractions, followed by secondary, longer-lasting and pronounced relaxations. The initial brief rises in the basal tones and amplitudes of isolated portal vein contractions have also been observed in studies involving quercetin (Chiwororo and Ojewole, unpublished observation). Myricetin has been reported to induce a biphasic effect on vascular tissues, exerting contractile effects at relatively low concentrations and relaxant effects at higher concentrations. The presence of three contiguous hydroxyl groups in the B ring potentiates, at low concentrations, vascular smooth muscle contractions induced by the
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myricetin (Herreira et al., 1996). In fact, potentiation of contractile responses exerted by flavonoids possessing three contiguous hydroxyl groups on either the A (5,6,7-trihydroxy), or B (3′,4′,5′-trihydroxy; myricetin) rings, has been observed previously in rat tail and femoral arteries to both transmural nerve stimulation-evoked, and exogenous agonist-provoked contractile responses of the vascular smooth muscles (Berger et al., 1992). Myricetin-induced potentiation of contractile responses to bath-applied agonists may be related to the activation of protein kinase C (Herreira et al., 1996). However, myricetin inhibits ATP-dependent Ca\(^{2+}\) transport system of rat liver plasma membrane (Thiyagarajah et al., 1991). This inhibition is thought to serve as a mechanism for augmenting or sustaining the elevation of intracellular Ca\(^{2+}\) that occurs following release of Ca\(^{2+}\) from intracellular stores (Chen and Van Breemen, 1993). However, if this claim also applies to vascular smooth muscles, then the myricetin-induced potentiation of contractile responses of vascular muscles to agonists could be attributed to inhibition of Ca\(^{2+}\)-ATPase activity (Herreira et al., 1996). In our study, however, pre-incubation of rat isolated portal veins with nifedipine only partially inhibited the initial brief contractile effects of PGE, suggesting that the flavonoids and other chemical constituents of the plant’s extract are unlikely to be acting on the Ca\(^{2+}\)-ATPase, or releasing Ca\(^{2+}\) from its stores in the vascular smooth muscles. In conclusion, the findings of this study indicate that Psidium guajava leaf aqueous extract exerts a biphasic effect on rat vascular smooth muscles, and support the use of the plant’s leaves as a natural, adjunct phytomedicine in the management of arterial hypertension.

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