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Inhibition of Intestinal Motility and Secretion by Flavonoids in Mice and Rats: Structure-activity Relationships

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Abstract—Intraperitoneal administration of some flavonoids (apigenin, flavone, kaempferol, morin, myricetin, naringin and rutin; 12·5–50 mg kg^{-1}) significantly ($P < 0{\cdot}05$–$0{\cdot}01$) reduced small (28–69%) and large (83–134%) intestinal transit in mice. Other flavonoids (naringenin, silibinin, silymarin and taxifolin, 100–200 mg kg^{-1}) reduced (23–41%; $P < 0{\cdot}5$–$0{\cdot}01$) intestinal transit at doses of 100–200 mg kg^{-1} while hesperitin, catechin and phloridzin (up to 200 mg kg^{-1}) had no effect. This effect was antagonized by yohimbine (87–96%) and phentolamine (87–91%) but not by prazosin, propranolol, atropine, hexamethonium, mepyramine, cyproheptadine and naloxone. Yohimbine (92–96%) also antagonized the inhibitory effect of flavonols (12·5–50 mg kg^{-1}) ($P < 0{\cdot}05$–$0{\cdot}01$) on intraluminal accumulation of fluid and diarrhoea induced by castor oil. By contrast, verapamil potentiated the flavonol effect. It is suggested that these effects, influenced by the structure of the molecules, are mediated by α_2-adrenergic receptors and calcium.

Flavonoids form a large class of phenolic substances widely distributed in nature. They have low toxicity in mammals and are used in medicine for maintenance of capillary integrity (Timberlake & Henry 1988).

However, flavonoids exhibit several biological effects such as aldose reductase and xanthine oxidase inhibition and anti-inflammatory, antihepatotoxic and anti-ulcer actions (Havsteen 1983; Pathak et al 1991). Flavonoids are also active on intestinal motility and recently it has been shown that quercetin, the most active flavonoid, inhibits contractions of guinea-pig ileum muscle stimulated either by agonists or anaphylaxis (Fanning et al 1983). Similar activity was also observed with certain other flavonoids (Middleton & Drzewiecki 1984; Capasso et al 1988, 1991).

Quercetin has also been found to inhibit guinea-pig ileum contractions induced by transmural electrical stimulation (Capasso et al 1991). It was concluded that certain flavonoids can exert a true spasmolytic effect on ileal smooth muscle. This was also the conclusion of Kazimierczak et al (1980) who showed a protective action of cromolyn sodium, a compound structurally related to flavonoids, against the contraction of guinea-pig ileum induced by various agonists. More recent studies have then shown that quercetin inhibits intestinal motility in-vivo and this effect seems potentiated by verapamil (Meli et al 1990), a calcium-channel blocker, and antagonized by yohimbine and phentolamine, α_2-adrenoceptor antagonists, suggesting that calcium and α_2-adrenergic receptors mediate the flavonoid-induced delay of small intestinal transit (Di Carlo et al 1993).

In the present study we have explored whether other flavonoids (apigenin, catechin, flavone, hesperitin, kaempferol, morin, myricetin, naringin, naringenin, phloridzin, rutin, silibinin, silymarin and taxifolin; Table 1, Fig. 1) delay intestinal transit in mice. We have also studied the effect of flavonoids and drugs (yohimbine, phentolamine, prazosin, atropine, hexamethonium, mepyramine, cyproheptadine, verapamil, naloxone and propranolol) on intraluminal fluid and sodium accumulation.

Because diarrhoea is a consequence of altered motility and fluid accumulation throughout the intestine, attempts also have been made to verify a possible antidiarrhoeal activity of flavonoids.

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Materials and Methods

Small intestinal transit

The effect of flavonoids on small intestinal transit was tested in male Swiss mice, 23–26 g, after a charcoal meal (Meli et al 1990). The animals were fasted for 3 h before intraperitoneal injection of flavonoid (12·5–200 mg kg^{-1}), but had free access to water. The charcoal meal, a suspension containing 10%

Table 1. Nomenclature of the subclasses of flavonoids according to their substituents.

	Substituents						
Flavonoids	3	5	7	2′	3′	4′	5′
Flavonols							
Kaempferol	OH	OH	OH	H	H	OH	H
Morin	OH	OH	OH	OH	H	OH	H
Rutin	O-Rh-Glc	OH	OH	H	OH	OH	H
Myricetin	OH	OH	OH	H	OH	OH	OH
Flavanones							
Hesperitin	H	OH	OH	H	OH	OCH_3	H
Naringin	H	OH	O-Rh	H	H	OH	H
Naringenin	H	OH	OH	H	H	OH	H
Flavanolols							
Silibinin	OH	OH	OH	H	H	OR*	OR*
Silymarin	OH	OH	OH	H	H	OR*	OR*
Taxifolin	OH	OH	OH	H	OH	OH	H
Flavones							
Apigenin	H	OH	H	OH	H	H	OH
Flavone	H	H	H	H	H	H	H
Flavan-3-ols							
Catechin	OH	OH	OH	H	OH	OH	H

*See Fig. 1; Rh = rhamnose; Glc = glucose.

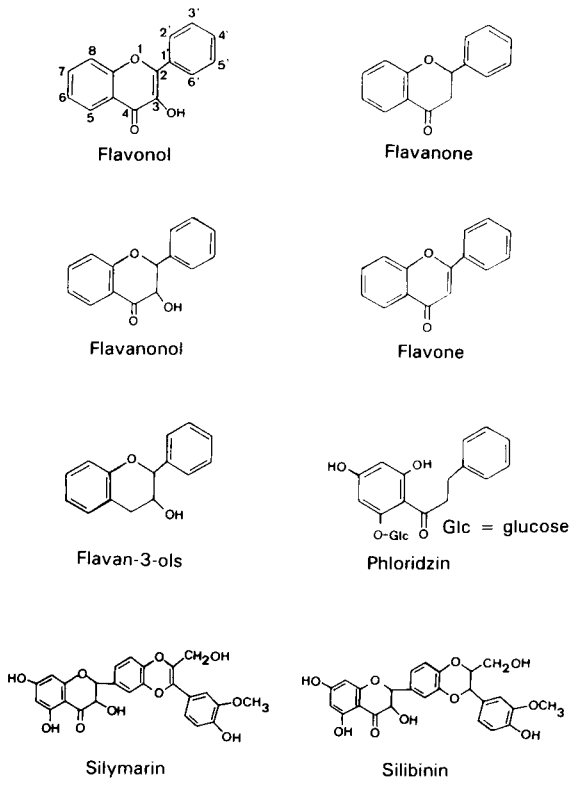

FIG. 1. Basic chemical structures of the different classes of flavonoids.

charcoal in 5% acacia gum (0·1 mL/10 g) was administered intragastrically by stomach tube 60 min after the intraperitoneal injection of flavonoid. Mice were killed after 20 min and the small intestine was rapidly removed and laid out on white filter paper for inspection and measurement of distances traversed by the charcoal. The distance traversed by the charcoal marker was calculated as a percentage of the intestine length. Control animals received vehicle (dimethylsulphoxide 0·05 mL/10 g body weight) for flavonoid.

To investigate the mechanism of action of flavonoids studied, different drugs acting on adrenergic, cholinergic, histaminergic, 5-hydroxytryptaminergic and opioid systems, and on calcium were used (mg kg^{-1}): yohimbine 1, phentolamine 1, prazosin 1, atropine 0·25, hexamethonium 5, propranolol 2·5, mepyramine 2·5, cyproheptadine 2·5, naloxone 10 and verapamil 5. These drugs were dissolved in 0·9% NaCl (saline) and given subcutaneously 10 min before flavonoid administration, with the exception of verapamil given intraperitoneally 30 min before the charcoal meal (Meli et al 1990). Control animals received vehicles for both flavonoid (dimethylsulphoxide 0·05 mL/10g) and the drug used (saline 0·05 mL/10 g).

Large intestinal transit

The effect of some flavonoids on large intestinal transit was measured in male Swiss mice, 35–40 g, after intracaecal administration of carmine red suspension (Di Carlo et al 1992). Animals were fasted for 3 h before intraperitoneal injection of flavonoid, but had free access to water. A suspension of carmine red (10 mg/0·2 mL water) was injected into the caecum through a chronically implanted (a week before) caecal catheter, and the time until appearance of the first coloured faeces was registered. Flavonoids (kaempferol, morin, rutin and myricetin; 25–50 mg kg^{-1}) were administered intraperitoneally 30 min before intracaecal marker application. In some experiments animals were treated with yohimbine (1 mg kg^{-1}) subcutaneously, or verapamil (5 mg kg^{-1}) intraperitoneally as described above. Control animals received vehicles only.

Intestinal fluid and electrolyte accumulation

Intraluminal fluid accumulation was determined by enteropooling (Robert et al 1976). Flavonoids (12·5–200 mg kg^{-1}) were administered intraperitoneally to male Wistar rats, 140–150 g, followed 1 h later by castor oil (2 mL). The animals were killed 30 min later, the entire small intestine removed, its contents collected and the volume measured. Samples of the fluid were analysed for Na^+ concentrations using an electrochemical method after HPLC (Poole & Schuette 1984). Some animals received yohimbine (1 mg kg^{-1}) subcutaneously or verapamil (5 mg kg^{-1}) intraperitoneally as described above.

Castor oil diarrhoea

Diarrhoea was induced by oral administration of castor oil to mice (0·2 mL) (Izzo et al 1992). Flavonoids (kaempferol, morin, myricetin and rutin; 25 and 50 mg kg^{-1}) were administered intraperitoneally 60 min before castor oil administration. Control mice received the same volume (0·05 mL/10 g) of dimethylsulphoxide intraperitoneally. Mice were scored (double blind) for copious (++), mild (+) or lack (0) of diarrhoea, 2 h after oil challenge. The activity score was calculated by taking the sum of the number of "+" rats and twice the number of "++" rats (Mascolo et al 1992). In some experiments animals were pretreated with yohimbine (1 mg kg^{-1}) subcutaneously or verapamil (5 mg kg^{-1}) intraperitoneally just before oil challenge.

Flavonoids used

Apigenin, catechin, hesperitin, flavone, kaempferol, morin, myricetin, naringin, naringenin, phloridzin, rutin and taxifolin were purchased from Sigma (Milan), while silibinin and silymarin were generous gifts from Madaus (Koln, Germany).

Drugs and other materials

Castor oil, yohimbine, propranolol, atropine sulphate, naloxone hydrochloride, hexamethonium, verapamil, mepyramine, cyproheptadine and dimethylsulphoxide were purchased from Sigma; prazosin hydrochloride from Pfizer (Milan), charcoal from Pharmacia (Naples), carmine red and acacia gum from local distributors.

Statistics

Diarrhoea was expressed as total score and the chi-square test was used to determine the significance between groups. Fluid volume, Na^+ secretion and intestinal transit were expressed as means ± s.e. and Student's *t*-test was used to determine the significance of difference between means. A P value less than 0·05 was taken as statistically significant.

Table 2. Effect of flavonoids on small intestinal transit, expressed as distance travelled by charcoal marker as % of total intestinal length. Flavonoids were given 60 min before a charcoal meal.

Flavonoids (mg kg^{-1})	12·5	25	50	100	200
Control	39·4 ± 4·1	39·4 ± 4·1	39·4 ± 4·1	39·4 ± 4·1	39·4 ± 4·1
Apigenin	34·3 ± 2·1	28·4 ± 2·0	23·7 ± 1·8^b	25·1 ± 1·5^b	24·7 ± 1·7^b
Catechin			35·1 ± 2·3	36·4 ± 2·3	35·8 ± 2·2
Flavone	35·1 ± 2·7	31·4 ± 2·6	23·1 ± 1·4^b	26·7 ± 1·4^b	25·4 ± 1·3^b
Hesperitin			37·1 ± 2·8	40·0 ± 3·1	39·1 ± 3·0
Kaempferol	28·1 ± 1·7^a	23·4 ± 1·9^b	14·1 ± 1·2^b	15·8 ± 1·0^b	16·1 ± 1·2^b
Morin	28·7 ± 1·8^a	26·1 ± 2·0^a	16·8 ± 1·4^b	17·3 ± 1·3^b	16·6 ± 1·7^b
Myricetin	29·4 ± 1·8^b	26·4 ± 3·0^b	15·9 ± 1·5^b	16·4 ± 2·0^b	15·8 ± 1·7^b
Naringin	36·8 ± 2·4	25·4 ± 2·1	22·8 ± 1·4^b	21·0 ± 1·4^b	22·4 ± 1·8^b
Naringenin	30·7 ± 2·0	31·8 ± 2·6	27·1 ± 3·3	23·0 ± 2·1^a	24·4 ± 2·0^a
Phloridzin			44·7 ± 3·6	37·1 ± 1·9	39·7 ± 2·1
Rutin	25·4 ± 1·9^a	16·7 ± 1·5^a	12·7 ± 1·0^b	13·0 ± 1·0^b	13·1 ± 1·0^b
Silibinin	33·7 ± 3·0	33·6 ± 2·4	30·4 ± 3·1	25·5 ± 2·3	24·4 ± 2·0^a
Silymarin	31·7 ± 3·1	30·4 ± 2·3	33·1 ± 1·9	24·2 ± 2·9^a	23·0 ± 2·1^b
Taxifolin			38·2 ± 3·0	30·7 ± 2·9	26·4 ± 2·5^a

Values are mean ± s.e. of 12 experiments (control group: 20 experiments). $^a P < 0·05$, $^b P < 0·01$ (Student's t-test).

Table 3. Effects of different drugs on the flavonoid-induced delay of small intestinal transit expressed as the distance traversed by the charcoal marker as a percentage of total intestinal length. Drugs were given 10 min before flavonoid administration with the exception of verapamil which was given 30 min after the flavonoid. Flavonoid (50 mg kg^{-1}) was given intraperitoneally 60 min before a charcoal meal.

Drug treatment	Dose (mg kg^{-1})	Control	Flavonoid treatment			
			Kaempferol	Morin	Rutin	Myricetin
None	—	40·1 ± 2·6	15·3 ± 1·4^a	17·0 ± 1·9^a	13·7 ± 1·2^a	18·8 ± 2·0^a
Yohimbine	1	38·8 ± 2·4	37·5 ± 2·7^b	38·4 ± 2·7^b	36·7 ± 2·7^b	39·1 ± 3·0^b
Phentolamine	1	41·0 ± 2·8	37·9 ± 3·0^b	38·1 ± 4·4^b	36·8 ± 2·7^b	37·9 ± 3·1^b
Prazosin	1	40·7 ± 2·3	16·1 ± 2·3	16·9 ± 2·0	14·0 ± 2·7	16·3 ± 2·0
Atropine	0·25	38·3 ± 2·3	14·1 ± 2·6	15·3 ± 2·0	12·7 ± 1·9	13·5 ± 2·0
Hexamethonium	5	42·0 ± 2·7	16·7 ± 1·8	16·9 ± 2·0	15·0 ± 2·0	17·0 ± 2·3
Mepyramine	2·5	38·3 ± 2·7	14·4 ± 1·9	15·4 ± 1·7	15·4 ± 1·8	15·8 ± 2·2
Cyproheptadine	2·5	40·4 ± 4·1	14·0 ± 2·0	16·8 ± 1·9	14·9 ± 1·9	16·4 ± 2·0
Verapamil	5	36·9 ± 4·0	6·9 ± 1·1^b	8·9 ± 1·3^b	6·4 ± 1·6^b	9·9 ± 1·9^b
Naloxone	10	38·6 ± 3·0	17·1 ± 1·7	16·4 ± 1·4	14·4 ± 1·4	17·3 ± 1·6
Propranolol	2·5	41·4 ± 3·4	14·6 ± 1·4	18·1 ± 2·0	14·1 ± 1·3	16·9 ± 1·7

Values are mean ± s.e. of 12 experiments. $^a P < 0·01$ vs control; $^b P < 0·01$ vs flavonoid (Student's t-test).

Results

Intestinal transit

Inhibition of small intestinal transit in mice by the 14 flavonoids studied is shown in Table 2. Catechin, hesperitin and phloridzin had no effect on intestinal transit at the highest dose used (200 mg kg^{-1}); the effects of naringenin, silibinin, silymarin and taxifolin, although evident at lower doses, became statistically significant only at 100–200 mg kg^{-1} (inhibition of transit 23–41% compared with vehicle control, $P < 0·05$–$0·01$). Apigenin, flavone and naringin were active at 25 mg kg^{-1} and the remaining four, kaempferol, morin, myricetin and rutin, at the lowest dose (12·5 mg kg^{-1}) used (up to 36% inhibition, $P < 0·05$). In all cases, any effect seen was maximal with the dose of 50 mg kg^{-1}, and increasing it beyond this had no further effect.

Pretreatment (Table 3) with yohimbine or phentolamine, but not with prazosin, atropine, hexamethonium, mepyramine, cyproheptadine, naloxone or propranolol, antagonized the effects of the flavonols kaempferol, morin, myricetin and rutin, whereas verapamil potentiated it.

Large intestinal transit

Transit through the large intestine was also significantly delayed by the flavonols (83–134%, $P < 0·05$–$0·01$). This effect increased with dose, but not linearly and was antagonized by yohimbine and increased by verapamil (Table 4).

Intestinal fluid and electrolyte secretion

Administration of castor oil increased 8- to 9-fold both fluid volume and sodium secretion from the intestine compared with untreated rats (Table 5). Both these effects were significantly reduced by pretreatment with varying doses of flavonoids (mg kg^{-1}). Catechin, hesperitin and phloridzin were inactive. In all cases the reduction was dose-dependent, but became significant only at the doses indicated in Table 5. Yohimbine (1 mg kg^{-1}) counteracted, and verapamil (5 mg kg^{-1}) potentiated the flavonoid effect (Table 6).

Castor oil diarrhoea

During the 2 h after castor oil administration, mice produced copious diarrhoea, the maximum score achieved being 28. The four flavonoids examined, kaempferol, morin, myricetin and rutin, reduced the score in a dose-related manner (Table 7). Yohimbine antagonized the effect of 50 mg kg^{-1} flavonoid, and verapamil potentiated that of 25 mg kg^{-1}.

Table 4. Effect of flavonoids on large intestinal transit, expressed as transit times (min).

Flavonoid	Dose (mg kg^{-1})	Treatment: None	Yohimbine (1 mg kg^{-1}) (subcutaneous)	Verapamil (5 mg kg^{-1}) (intraperitoneal)
Control		47 ± 10	44 ± 12	43 ± 11
Kaempferol	25	88 ± 10[a]	48 ± 13[c]	93 ± 12
	50	99 ± 10[b]	52 ± 17[d]	143 ± 11[c]
Morin	25	88 ± 11[a]	66 ± 9	100 ± 9
	50	98 ± 17[b]	51 ± 10[d]	137 ± 13[c]
Rutin	25	90 ± 12[a]	41 ± 8[c]	104 ± 10
	50	110 ± 17[b]	56 ± 13[d]	171 ± 15[c]
Myricetin	25	86 ± 10[a]	47 ± 11[c]	99 ± 12
	50	95 ± 15[b]	49 ± 13[d]	141 ± 14[c]

[a] $P < 0.05$, [b] $P < 0.01$ vs control; [c] $P < 0.05$, [d] $P < 0.01$ vs flavonoid alone.

Table 5. Inhibition of castor oil stimulated fluid and Na^+ secretion in rat small intestine by flavonoids.

Treatment	Dose (mg kg^{-1}) (intraperitoneal)	Volume (mL)	Na^+ (μmol)
Normal (no castor oil)		0·22 ± 0·05	26·4 ± 3·7
Control (no flavonoid)		1·61 ± 0·13	231·4 ± 16·4
Apigenin	50	1·34 ± 0·12	179·3 ± 11·4
	100	0·93 ± 0·11[a]	77·4 ± 6·4[b]
Catechin	50	1·59 ± 0·16	233·4 ± 19·1
	100	1·56 ± 0·16	230·5 ± 18·7
Flavone	50	1·31 ± 0·14	171·4 ± 10·4[a]
	100	0·95 ± 0·13[a]	73·8 ± 6·9[b]
Hesperitin	50	1·61 ± 0·16	227·6 ± 20·1
	100	1·60 ± 0·14	223·5 ± 17·4
Kaempferol	50	1·10 ± 0·09[a]	159·8 ± 12·0[a]
	100	0·60 ± 0·08[b]	41·3 ± 5·9[b]
Morin	12·5	1·27 ± 0·16	180·3· ± 13·1
	50	0·66 ± 0·09[a]	47·3 ± 5·8[a]
Myricetin	12·5	1·19 ± 0·14	180·1 ± 13·0
	50	0·55 ± 0·09[a]	44·5 ± 5·7[b]
Naringin	50	1·40 ± 0·13	180·1 ± 11·1
	100	0·91 ± 0·11[a]	75·6 ± 8·0[b]
Naringenin	50	1·44 ± 0·15	220·7 ± 15·7
	100	1·00 ± 0·16[a]	151·7 ± 13·0[a]
Phloridzin	50	1·61 ± 0·17	227·3 ± 20·1
	100	1·55 ± 0·15	230·5 ± 19·0
Rutin	12·5	1·06 ± 0·10[a]	168··6 ± 13·4[a]
	50	0·50 ± 0·07[a]	39·4 ± 5·7[b]
Silibinin	50	1·48 ± 0·16	217·4 ± 10·8
	200	1·08 ± 0·13[a]	150·3 ± 11·4[a]
Silymarin	50	1·44 ± 0·16	213·1 ± 10·9
	200	1·05 ± 0·10[a]	157·4 ± 13·0[a]
Taxifolin	50	1·34 ± 0·13	220·7 ± 16·0
	200	1·04 ± 0·11[a]	158·3 ± 12·7[a]

Results are expressed as means ± s.e. of 10–12 experiments. [a] $P < 0.05$, [b] $P < 0.01$ vs control.

Discussion

The inhibitory effect of flavonoids on gastrointestinal functions, motility and secretion, was influenced to a great extent by the structure of the molecules (Fig. 1, Table 1), the effect of flavanol being diminished or abolished by absence of the 3-hydroxyl group (apigenin, flavone), saturation of the C-2, C-3 double bond (silibinin, taxifolin), saturation of the C-2, C-3 double bond and removal of the 3-hydroxyl group (naringenin, naringin) and the presence of a 4′-methoxyl group (hesperitin), cyclization of 4′,5′-hydroxyl groups (silibinin, silymarin), lack of the C-4 carbonyl (catechin) or opening of the B ring (phloridzin). Glycosylation increased the biological activity of compounds; naringin (glycosylation

Table 6. Effects of verapamil and yohimbine on the inhibitory action of flavonoids on fluid and sodium secretion in small intestine of rats treated with castor oil. All flavonoids were given intraperitoneally 60 min before castor oil (2 mL). Verapamil (5 mg kg^{-1}) intraperitoneally and yohimbine (1 mg kg^{-1}) subcutaneously, were given 10 min before flavonoids.

Treatment	Flavonoid dose (mg kg^{-1})	Fluid volume (mL)	Na^+ (μmol)
Control		1·61 ± 0·13	231·4 ± 16·4
Verapamil alone		1·64 ± 0·13	247·1 ± 17·4
Yohimbine alone		1·58 ± 0·15	237·4 ± 0·17
Kaempferol	25	0·97 ± 0·10[a]	111·1 ± 10·1[a]
+ verapamil	25	0·47 ± 0·07[c]	40·3 ± 5·1[d]
Kaempferol	50	0·58 ± 0·07[b]	44·6 ± 6·0[b]
+ yohimbine	50	1·44 ± 0·13[d]	223·2 ± 14·4[d]
Morin	25	0·96 ± 0·09[a]	109·4 ± 9·7[b]
+ verapamil	25	0·45 ± 0·06[c]	39·4 ± 5·0[d]
Morin	50	0·58 ± 0·10[b]	49·9 ± 5·8[b]
+ yohimbine	50	1·51 ± 0·14[d]	230·7 ± 13·4[d]
Myricetin	25	1·00 ± 0·11[a]	118·4 ± 10·9[a]
+ verapamil	25	0·43 ± 0·06[c]	37·4 ± 4·9[d]
Myricetin	50	0·55 ± 0·10[b]	47·8 ± 6·9[b]
+ yohimbine	50	1·45 ± 0·16[d]	220·4 ± 0·14[d]
Rutin	25	0·87 ± 0·09[a]	100·1 ± 9·3[a]
+ verapamil	25	0·39 ± 0·05[c]	35·8 ± 4·2[d]
Rutin	50	0·48 ± 0·05[b]	40·1 ± 5·8[b]
+ yohimbine	50	1·47 ± 0·12[d]	220·7 ± 16·8[d]

Results are expressed as mean ± s.e. of 7–9 experiments. [a] $P < 0·05$, [b] $P < 0·01$ vs control; [c] $P < 0·05$, [d] $P < 0·001$ vs flavonoid alone (Student's t-test).

Table 7. Diarrhoea induced in mice by castor oil (0·2 mL): effects of flavonoids without and with verapamil or yohimbine. All flavonoids were given intraperitoneally 60 min before castor oil. Verapamil (5 mg kg^{-1}) intraperitoneally and yohimbine (1 mg kg^{-1}) subcutaneously, were given 10 min before flavonoid.

Treatment	Dose (mg kg^{-1})	Diarrhoea score ++	+	0	Total score Max = 28
Control		14	0	0	28
Verapamil alone		13	1	0	27
Yohimbine alone		14	0	0	28
Kaempferol	25	5	2	7	12[a]
+ verapamil	25	1	0	13	2[c]
Kaempferol	50	2	2	10	6[b]
+ yohimbine	50	10	3	1	23[c]
Morin	25	6	1	7	13[a]
+ verapamil	25	1	0	13	2[c]
Morin	50	2	1	11	5[b]
+ yohimbine	50	12	2	0	26[c]
Myricetin	25	7	0	7	14[a]
+ verapamil	25	1	0	13	2[c]
Myricetin	50	2	2	10	6[b]
+ yohimbine	50	11	3	0	25[c]
Rutin	25	3	4	7	10[a]
+ verapamil	25	0	1	12	1[c]
Rutin	50	0	3	11	3[b]
+ yohimbine	50	12	0	2	24[c]

[a] $P < 0·05$, [b] $P < 0·01$ vs control; [c] $P < 0·01$ vs flavonoid alone (chi square analysis); 14 animals in each group.

at C-7) was more active than naringenin and glycosylation at C-3 (rutin) increased further the flavonoid effect.

It has been proposed that the α_2-agonists, clonidine and xylazine, inhibit intestinal transit (Ruwart et al 1980; Hsu 1982) and clonidine has recently been proposed as an antidiarrhoeal drug (Brown 1991). Our results show that the α_2-adrenoceptor antagonists yohimbine and phentolamine counteracted the effects of flavonoids on intestinal transit, while prazosin, an α_1-adrenoceptor antagonist (Cambridge et al 1977) and propranolol, a β-adrenoceptor antagonist, failed to do so. This indicates a role for the α_2-adrenergic system in the action of the flavonoids examined.

Several studies indicate an interaction between α_2-adrenergic and opioid systems (Atlas & Sabol 1980; Browning et al 1982; Ramaswamy et al 1983; Viswanathan et al 1984a), and it has been reported that the flavonoid gossypin possesses an opioid-mediated analgesic effect (Viswanathan et al 1984b). Interactions between adrenergic and cholinergic systems have also been reported (Vizi & Knoll 1971; Vizi 1979).

In the present study the effect of flavonoids on intestinal motility was unaffected by naloxone, atropine or hexamethonium. Further, histaminergic (mepyramine) and 5-hydroxytryptaminergic (cyproheptadine) antagonists did not modify the action of flavonoids on intestinal transit, indicating that this action did not involve opioid, cholinergic, histaminergic or 5-hydroxytryptaminergic mediation.

Our results also show that intraluminal fluid accumulation and diarrhoea induced by castor oil were inhibited by flavonoids in a dose-related manner. This effect was inhibited by yohimbine, indicating a role for the α_2-adrenoceptor in secretion, as well as in gut motility.

Based on the use of the calcium-channel antagonists verapamil or nifedipine, a calcium-mediated mechanism has recently been suggested to explain the spasmolytic effects of flavonoids and other compounds on the gastrointestinal tract (Capasso et al 1991). Calcium also regulates the balance between absorption and secretion across the intestinal mucosa (Berridge 1984); a low intracellular calcium concentration favours absorption, whereas a rise in intracellular calcium promotes secretion (Donowitz 1983).

Loperamide, an antidiarrhoeal drug, increases intestinal transit time and reduces intestinal secretion through a blockade of calcium channels (Reynolds et al 1984). Our data show that blockage of calcium channels by verapamil reinforces the inhibitory effect of some flavonoids on motility, secretion and diarrhoea.

In conclusion, the present results suggest that flavonoids produce an inhibitory effect on intestinal functions, and that their action is mediated through α_2-adrenergic and calcium systems.

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