Influence of Anti-Inflammatory Flavonoids on Degranulation and Arachidonic Acid Release in Rat Neutrophils

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We assessed the effects of 24 flavonoid derivatives, reported as anti-inflammatory, on lysosomal enzyme secretion and arachidonic acid release in rat neutrophils. Amentoflavone, quercetagetin-7-O-glucoside, apigenin, fisetin, kaempferol, luteolin and quercetin were the most potent inhibitors of β-glucuronidase and lysozyme release. The first compound was also able to inhibit basal release. These flavonoids besides chrysin and to a reduced extent, naringenin, significantly inhibited arachidonic acid release from membranes. A correlation between degranulation and arachidonic acid release was found for this series of compounds. Structure-activity relationships and implications for the anti-inflammatory effects of these flavonoids were discussed.

Introduction

Besides a wide range of biological activities (Havsteen, 1983) some members of the flavonoid class display anti-inflammatory effects in animals (Alcaraz and Jiménez, 1988; Ferrándiz and Alcaraz, 1991). The mechanism of action is unclear but it may involve the inhibition of eicosanoids production by intact cells with different selectivity towards cyclo-oxygenase or lipoxygenase pathways depending on their structural features (Moroney et al., 1988). Other actions possibly account for the molecular mechanisms leading to control the inflammatory process. As suggested for many nonsteroidal anti-inflammatory agents (Smolen and Weissmann, 1993; Kaplan et al., 1984; Neal et al., 1987; Smolen et al., 1991; Inoue et al., 1991; Zimmerli et al., 1991) the suppression of leukocyte functions may provide a basis for the anti-inflammatory effects of flavonoids. In this respect, recent attention has focused upon the inhibitory effects of flavonoids on reactive oxygen species generation in neutrophils (Limasset et al., 1993).

Secretion is a crucial event induced by leukocyte activation and resulting in the release of degradative enzymes which play an important role in tissue damage during inflammation. There are several possible links between arachidonic acid release and secretion. Thus, products derived from PLA_2 activity could be required for the fusion of lysosomes and plasma membranes, or arachidonic acid released would induce degranulation and other neutrophil responses (Smolen and Weissmann, 1993).

The objective of this study was to obtain evidence as to whether a series of flavonoids endowed with anti-inflammatory properties, modify selected neutrophil responses which contribute to the inflammatory process. Thus, we have investigated the capacity of a series of flavonoids to inhibit degranulation in rat neutrophils and we have included as reference some compounds, like quercetin, previously reported as inhibitors of leukocyte functions in other species (Bennett et al., 1981; Middleton Jr. et al., 1987; Showell et al., 1981; Blackburn et al., 1987; Berton et al., 1980). In order to understand better the modification of eicosanoid generation by flavonoids in relevant inflammatory cells, we have also assessed in the present study the influence of these flavonoids on the release of arachidonic acid from leukocyte membranes, a process mainly dependent on PLA_2 activity.

Materials and Methods

Drugs

Sideritoflavone was isolated from *Sideritis leucantha* and hypolaetin-8-O-glucoside from *Sideritis mugronensis*, following known procedures (Villar *et al.*, 1985; Jiménez *et al.*, 1986). Oroxindin from *Oroxylum indicum;* quercetagetin-7-O-glucoside from *Tagetes erecta,* gossypin and hibifolin from *Hibiscus vitifolius,* and tambuletin from *Zhanthoxylum alatum* were a gift from Prof. A. G. R. Nair (Pondicherry University, India). Other flavonoids were commercially available: flavone, 3-hydroxyflavone and fisetin (Aldrich); troxerutin (Almirall); quercetin (Merck); apigenin, luteolin and amentoflavone (Roth); leucocyanidol (Rovi); chrysin, rutin, morin, naringenin, naringin, (+)-catechin, (−)-epicatechin and kaempferol (Sigma). [^{14}C]-arachidonic acid (100 µCi/ml) was purchased from DuPont. Ionophore A 23187, BSA, glycogen, cytochalasin B, FMLP and triton X-100 were purchased from Sigma. The rest of the chemicals were of analytical grade.

Isolation of leukocytes

Rat peritoneal leukocytes elicited by glycogen (10 ml, 1%) were prepared by centrifugation and hypotonic lysis of contaminating red cells (Moroney *et al.*, 1988). The cell suspension contained 90% neutrophils and viability was greater than 95% assessed by Trypan blue exclusion.

Lysosomal enzyme secretion

Leukocytes were suspended at 6×10^6 cells/ml in PBS and preincubated for 8 min at 37 °C with cytochalasin B (5 µg/ml) followed by addition of test compound or vehicle (10 µl DMSO). After 8 min, FMLP (10^{-6} M) was added and incubation proceeded for 8 min. The cell suspensions were then placed on ice and centrifuged at 3000 r.p.m. for 15 min at 4 °C. β-Glucuronidase and lysozyme levels in supernatants were determined by spectrophotometric procedures previously described (Gianetto and de Duve, 1955; Yuli *et al.*, 1982) and results were expressed as percentage of release with respect to tubes treated with 10 µl 20% triton X-100.

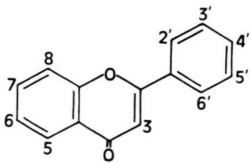### Arachidonic acid release (Croft et al., 1987)

Cells were suspended in Hank's solution at a concentration of 10^7 ml and incubated with arachidonic acid (0.1 µCi/ml) for 60 min at 37 °C. Leukocytes were then washed in PBS three times and resuspended in Hank's solution + 0.1% BSA. Test compounds were preincubated for 15 min at 37 °C before addition of ionophore A 23187 (25 µg/ml). After 10 min, tubes were placed on ice and centrifuged as above. Radioactivity was determined in supernatants by liquid scintillation counting.

Results were expressed as means ± SE for 3−6 determinations. Compounds able to inhibit control reactions at least by 50% were tested over a range of concentrations to determine their half-maximal inhibitory concentration. Statistical analysis was performed by using the Dunnett's t-test.
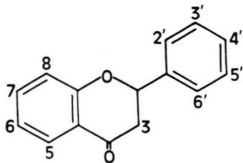
Results

In control incubations, the extent of lysozyme secretion was more than twice that of β-glucuronidase either in the presence or in the absence of stimulus (data not shown). A number of flavonoids inhibited β-glucuronidase and lysozyme release, taken as markers of rat peritoneal leukocytes degranulation (Table I). Amentoflavone, quercetagetin-7-O-glucoside, apigenin, fisetin, kaempferol, luteolin and quercetin yielded more than 60% of inhibition on both enzymes secretion. Besides, oroxindin, chrysin and hibifolin affected selectively β-glucuronidase release. The regression analysis of the curves obtained for these active compounds, showing more than 60% of inhibition at 10^{-4} M, at a range of concentrations between 10^{-5} M and 10^{-4} M (or 10^{-6} M and 10^{-4} M for amentoflavone), allowed us the calculation of the inhibitory concentration 50% (IC_{50}). Amentoflavone showed the highest potency, especially for inhibition of β-glucuronidase release, with an IC_{50} in the µM range (Fig. 1). At concentrations between 3×10^{-5} M and 10^{-4} M amentoflavone gave percentages of inhibition for β-glucuronidase release higher than 100%, indicating its ability to influence degranulation in basal conditions (in the absence of any stimulus). In this case, its IC_{50} was $4.5 \pm 0.1 \times 10^{-5}$ M and $6.2 \pm 0.4 \times 10^{-4}$ M, for β-glucuronidase and lysozyme basal release, respectively. For most com-

Table I. Structure of the flavonoids tested. Effect on leukocyte degranulation: percentage of inhibition at 10^{-4} M (% I) and inhibitory concentration 50% (IC_{50}).
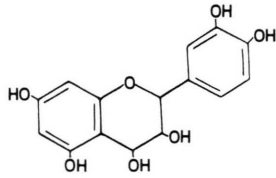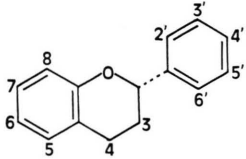
vones	R_5	R_6	R_7	R_8	$R_{3'}$	$R_{4'}$	β-Glucuronidase % I	IC_{50} ($\times 10^{-5}$ M)	Lysozyme % I	IC_{50} ($\times 10^{-5}$ M)
vone	H	H	H	H	H	H	56.7 ± 2.9**	N.D.	32.6 ± 1.0**	N.D.
rysin	OH	H	OH	H	H	H	72.0 ± 4.8**	6.6 ± 0.9	51.7 ± 3.3**	N.D.
igenin	OH	H	OH	H	H	OH	92.9 ± 4.0**	2.6 ± 0.3	71.8 ± 3.3**	6.5 ± 0.7
eolin	OH	H	OH	H	OH	OH	90.9 ± 3.0**	3.5 ± 1.2	72.0 ± 1.3**	4.6 ± 0.3
eritoflavone	OH	OCH_3	OCH_3	OCH_3	OH	OH	32.7 ± 2.1**	N.D.	11.0 ± 5.9	N.D.
polaetin-8-O-Gl	OH	H	OH	OGl	OH	OH	2.0 ± 2.2	N.D.	12.2 ± 2.4	N.D.
oxindin	OH	H	OGlr	OCH_3	H	H	70.8 ± 3.1**	7.4 ± 1.1	44.3 ± 2.0**	N.D.

vonols	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	β-Glucuronidase % I	IC_{50} ($\times 10^{-5}$ M)	Lysozyme % I	IC_{50} ($\times 10^{-5}$ M)
lydroxyflavone	OH	H	H	H	H	H	H	H	−103.2 ± 2.1**	6.7 ± 0.7	−54.2 ± 6.5**	N.D.
etin	OH	H	H	OH	H	H	OH	OH	100.0 ± 5.2**	2.3 ± 0.3	101.7 ± 14.2**	3.3 ± 0.2
empferol	OH	OH	H	OH	H	H	H	OH	74.4 ± 3.7**	4.5 ± 1.2	75.8 ± 8.9**	2.9 ± 0.8
ercetin	OH	OH	H	OH	H	H	OH	OH	90.4 ± 0.9**	2.4 ± 0.1	88.5 ± 2.7**	2.1 ± 0.2
orin	OH	OH	H	OH	H	OH	H	OH	36.3 ± 7.0**	N.D.	0.7 ± 3.3	N.D.
ercetagetin-7-O-Gl	OH	OH	OH	OGl	H	H	OH	OH	72.4 ± 2.7**	6.9 ± 1.2	74.7 ± 6.2**	4.9 ± 1.0
mbuletin	OH	OH	H	OCH_3	OGl	H	OH	OCH_3	13.8 ± 2.8*	N.D.	41.4 ± 15.0**	N.D.
tin	ORu	OH	H	OH	H	H	OH	OH	35.1 ± 11.9*	N.D.	12.3 ± 11.2	N.D.
oxerutin	ORu	OH	H	OHE	H	H	OHE	OHE	36.9 ± 4.0	N.D.	38.3 ± 2.1	N.D.
ssypin	OH	OH	H	OH	OGl	H	OH	OH	15.5 ± 1.8	N.D.	0.5 ± 10.3	N.D.
pifolin	OH	OH	H	OH	OGlr	H	OH	OH	62.4 ± 4.0**	7.1 ± 1.0	9.3 ± 6.2	N.D.

avanones	R_5	R_7	$R_{3'}$	$R_{4'}$	β-Glucuronidase % I	IC_{50} ($\times 10^{-5}$ M)	Lysozyme % I	IC_{50} ($\times 10^{-5}$ M)
ringenin	OH	OH	H	OH	23.6 ± 4.8**	N.D.	8.0 ± 2.0	N.D.
ringin	OH	ORu	H	OH	−1.9 ± 1.6	N.D.	−5.5 ± 7.5	N.D.

Leucocyanidol

avanols	R_3	R_4	R_5	R_7	$R_{3'}$	$R_{4'}$	β-Glucuronidase % I	IC_{50} ($\times 10^{-5}$ M)	Lysozyme % I	IC_{50} ($\times 10^{-5}$ M)
)-Catechin	SOH	H, H	OH	OH	OH	OH	−7.5 ± 1.4	N.D.	−4.4 ± 3.7	N.D.
)-Epicatechin	ROH	H, H	OH	OH	OH	OH	−4.2 ± 2.5	N.D.	10.2 ± 10.9	N.D.
ucocyanidol							14.2 ± 1.2*	N.D.	25.6 ± 2.3	N.D.

Biflavones	β-Glucuronidase		Lysozyme	
	% I	IC$_{50}$ ($\times 10^{-5}$ M)	% I	IC$_{50}$ ($\times 10$
Amentoflavone	131.6 ± 2.4**	0.57 ± 0.0	86.0 ± 3.5**	2.5 ± 0.3
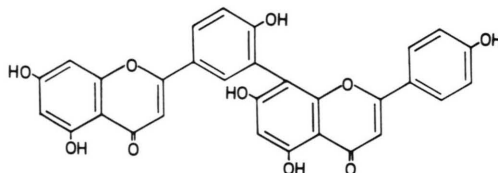
Gl = Glucose, Ru = Rutinose, Glr = Glucuronic acid, HE = Hydroxyethyl. * P < 0.05, ** P < 0.01. The enzyme release in control tubes was 28.9 ± 1.7 and 67.5 ± 1.9 (mean $\pm$ SE, n = 30) % of the total enzyme content in cells, for β-glucuronidase and lysozyme, respectively.
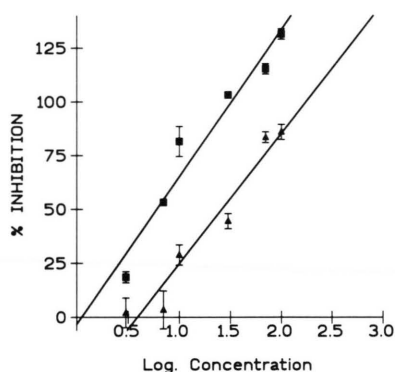

Fig. 1. Regression lines of amentoflavone (log. µM concentrations) for inhibition of β-glucuronidase (squares) and lysozyme (triangles) release. All points were statistically significant at least at P < 0.05, except for the two lower concentrations of amentoflavone on lysozyme release.

pounds the potency exhibited for inhibition of the two enzymic markers was similar.

3-Hydroxyflavone was the only compound which exhibited a biphasic effect, since it inhibited β-glucuronidase release without influencing lysozyme release, but at higher concentrations it stimulated degranulation in a significant manner (103.2 ± 2.1 and 54.2 ± 6.5% increase at 10^{-4} M, for β-glucuronidase and lysozyme release, respectively, P < 0.01). In another set of experiments carried out in the absence of any stimulus, 3-hydroxyflavone dose-dependently increased β-glucuronidase and lysozyme release, with 282.8 ± 1.5 and 139.5 ± 12.4% increase respectively (P < 0.01), at the concentration of 10^{-4} M. Concentrations causing approximately 50% increase with respect

to blank samples (without flavonoid) were 7.0×10^{-5} M and 3.0×10^{-5} M, respectively.

The flavonols fisetin, kaempferol and quercetagetin-7-O-glucoside, the flavones chrysin, apigenin and luteolin, as well as amentoflavone and to a very reduced extent, naringenin, significantly inhibited arachidonic acid release (Fig. 2). The reference compound, quercetin needed a concentration higher than those able to inhibit purified PLA$_2$ activity of different origins (Fawzy *et al.*, 1988). We also found a significant correlation between inhibition of β-glucuronidase secretion and of arachidonic acid release (r = 0.73, P < 0.001), as well as between inhibition of lysozyme secretion and of arachidonic acid release (r = 0.82, P < 0.001), for the series of flavonoids studied.
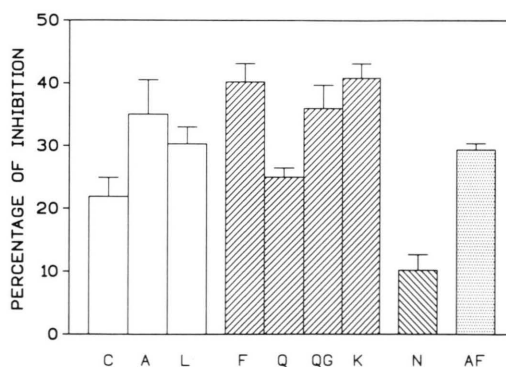

Fig. 2. Effect of flavonoids tested on arachidonic acid release. C = chrysin, A = apigenin, L = luteolin, F = fisetin, Q = Quercetin, QG = quercetagetin-7-O-glucoside, K = kaempferol, N = naringenin and AF = amentoflavone. All results were significant at P < 0.01, except for naringenin (P < 0.05).

Discussion

In our experiments lysozyme release levels were higher than β-glucuronidase release levels as reported by some authors in other systems (Ward *et al.*, 1984; Cockcroft and Stutchfield, 1989). This was probably due to a higher sensitivity of specific granules against different stimuli. This fact could explain that some flavonoids have been more effective on azurophilic granules secretion.

Quercetin has been included in our study as a reference compound since its inhibitory effects on lysosomal enzyme release have been demonstrated in a number of systems such as rabbit neutrophils (Bennett *et al.*, 1981; Showell *et al.*, 1981) and human neutrophils (Blackburn *et al.*, 1987) stimulated by FMLP or concanavalin A (Berton *et al.*, 1980). Our results are in accordance with those previously reported for quercetin, apigenin and luteolin on β-glucuronidase release in human neutrophils (Middleton *et al.*, 1987), although we have used a different cell system. We have also obtained a better potency for flavone and a similar effect for 3-hydroxyflavone, with respect to results reported in rabbit neutrophils (Bennett *et al.*, 1981).

Hypolaetin-8-O-glucoside failed to significantly affect lysosomal enzyme secretion. This is not surprising since many glycosides show a low level of activity on *in vitro* systems. Nevertheless, in an experimental model of inflammation in rats, we previously demonstrated that this flavonoid inhibited β-glucuronidase release (Villar *et al.*, 1987).

The flavonoids able to inhibit lysosomal enzyme release share some structural features. They are polyhydroxylated aglycones of the flavone or flavonol types and the presence of a free hydroxyl group at C4' increases the inhibitory activity (chrysin-apigenin). Other important features are the keto group at 4 position and the C2−C3 double bond. In contrast, the glycosylation (quercetin-rutin, naringenin-naringin) or the introduction of a free hydroxyl at C2' (kaempferol-morin) seems to be detrimental. In summary, the most active compounds were C2−C3 unsaturated, hydroxylated at C7 and C4' and with some additional hydroxyl groups (at C5, C3 or C3'), characteristics included in those found for inhibition of other leukocyte functions such as reactive oxygen species production by human neutrophils stimulated by FMLP (Limasset *et al.*, 1993).

Flavonoids have been less effective on arachidonic acid release than on lysosomal enzyme secretion; nevertheless, the structural features related to their inhibitory activity are similar for both neutrophil responses.

The precise mechanism of flavonoid inhibition of leukocyte functions is unclear. Recently, it has been suggested a possible interaction of the less hydrophilic flavonoids with the lipid environment of the membrane FMLP receptor or with the receptor itself, while compounds with at least four hydroxyl groups would act at a later stage of the activation process and thus they would influence leukocyte responses in a non-specific way (Limasset *et al.*, 1993). However, interaction of polyhydroxylated flavonoids with membranes has been reported (Ratty *et al.*, 1988) and it has been related to the protective effects of these compounds on different cell types (Perrissoud and Testa, 1986). It is interesting to note that the inhibition of PLA_2 activity by quercetin has been explained in part by its ability to interact with phospholipidic substrates (Fawzy *et al.*, 1988). In addition, the behaviour of amentoflavone which decreases basal secretion, suggests a non-specific stabilizing action on cell membranes by this flavonoid, while 3-hydroxyflavone could have the opposite effect, partially masked in the presence of stimulus due to some likely interference with the activation pathway.

We have demonstrated that this series of anti-inflammatory flavonoids can affect neutrophil function through mechanisms not attributable to their effects on either the cyclo-oxygenase or lipoxygenase pathway. Flavonoids have not exhibited a high potency on these *in vitro* tests, nevertheless high doses of flavonoids are usually needed to exert inhibitory effects on *in vivo* models of inflammation (Villar *et al.*, 1987; Alcaraz and Jiménez, 1988; Ferrándiz and Alcaraz, 1991) where they may achieve a range of concentrations enough to interact with leukocyte functions. Thus, with the limitations derived from an *in vitro* study, it is likely that quercetagetin-7-O-glucoside, apigenin, fisetin, kaempferol, luteolin, quercetin and especially, amentoflavone may, at least in part, affect the inflammatory response through their action on neutrophils.

Alcaraz M. J. and Jiménez M. J. (1988), Flavonoids as anti-inflammatory agents. Fitoterapia **59**, 25−38.

Bennett J. P., Gomperts B. D. and Wollenweber E. (1981), Inhibitory effects of natural flavonoids on secretion from mast cells and neutrophils. Arzneim. Forsch. **31 (I)**, 433−437.

Berton G., Schneier C. and Romeo D. (1980), Inhibition by quercetin of activation of polymorphonuclear leucocyte functions. Stimulus-specific effects. Biochim. Biophys. Acta **595**, 47−55.

Blackburn W. D., Heck L. W. and Wallace R. W. (1987), The bioflavonoid quercetin inhibits neutrophil degranulation, superoxide production, and the phosphorylation of specific neutrophil proteins. Biochem. Biophys. Res. Comms. **144**, 1229−1236.

Cockcroft S. and Stutchfield J. (1989), The receptors for ATP and fMetLeuPhe are independently coupled to phospholipases C and A_2 *via* G-protein(s). Relationship between phospholipase C and A_2 activation and exocytosis in HL60 cells and human neutrophils. Biochem. J. **263**, 715−723.

Croft K. D., Beilin L. J. and Ford G. L. (1987), Differential inhibition of thromboxane B_2 and leukotriene B_4 biosynthesis by two naturally occurring acetylenic fatty acids. Biochim. Biophys. Acta **921**, 621−624.

Fawzy A. A., Vishwanath B. S. and Franson R. C. (1988), Inhibition of human non-pancreatic phospholipases A_2 by retinoids and flavonoids-mechanism of action. Agents Actions **25**, 394−400.

Ferrándiz M. L. and Alcaraz M. J. (1991), Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. Agents Actions **32**, 283−286.

Gianetto R. and de Duve C. (1955), Comparative study of the binding of acid phosphatase, β-glucuronidase and cathepsin by rat-liver particles. Biochemistry **59**, 433−438.

Havsteen B. (1983), Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. **32**, 1141−1148.

Inoue K., Fujisawa H., Sasaki Y., Nishimura T., Nishimura I., Inoue Y., Yokota M., Masuda T., Ueda F., Shibata Y., Kimura K., Komiya Y. and Nishioka J. (1991), Pharmacological properties of the new nonsteroidal anti-inflammatory agent etodolac. Arzneim. Forsch. **41 (I)**, 228−235.

Jiménez M. J., Alcaraz M. J., Ferrándiz M. L. and Villar A. (1986), Anti-inflammatory activity of a flavone from *Sideritis leucantha,* Planta Med. **52**, 541.

Kaplan H. B., Edelson H. S., Korchak H. M., Given W. P., Abramson S. and Weissmann G. (1984), Effects of non-steroidal anti-inflammatory agents on human neutrophil functions *in vitro* and *in vivo*. Biochem. Pharmacol. **33**, 371−378.

Limasset B., Le Doucen C., Dore J.-C., Ojasoo T., Damon M. and Crastes de Paulet A. (1993), Effects of flavonoids on the release of reactive oxygen species by stimulated human neutrophils. Multivariate analysis of structure-activity relationships (SAR). Biochem. Pharmacol. **46**, 1257−1271.

Middleton E. Jr., Drzewiecki G. and Tatum J. (1987), The effects of Citrus flavonoids on human basophil and neutrophil function. Planta Med. **53**, 325−328.

Moroney M. A., Alcaraz M. J., Forder R. A., Carey F. and Hoult J. R. S. (1988), Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavonoid glycoside and related aglycone flavonoids. J. Pharm. Pharmacol. **40**, 787−792.

Neal T. M., Vissers M. C. M. and Winterbourn C. C. (1987), Inhibition by non-steroidal anti-inflammatory drugs of superoxide production and granule enzyme release by polymorphonuclear leukocytes stimulated with immune complexes or formyl-methionyl-leucyl-phenylalanine. Biochem. Pharmacol. **36**, 2511−2517.

Perrissoud D. and Testa B. (1986), Inhibiting or potentiating effects of flavonoids on carbon tetrachloride-induced toxicity in isolated rat hepatocytes. Arzneim. Forsch. **36 (II)**, 1249−1253.

Ratty A. K., Sunamoto J. and Das N. P. (1988), Interaction of flavonoids with 1,1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase. Biochem. Pharmacol. **37**, 989−995.

Showell H. J., Naccache P. H., Walenga R. W., Dalecki M., Feinstein M. B., Sha'afi R. I. and Becker E. L. (1981), The effects of quercetin, 1-tosylamido-2-phenylethyl chloromethyl ketone, cytochalasin A and nordihydroguaiaretic acid on lysosomal enzyme secretion, arachidonic acid metabolism and Ca^{2+} fluxes in rabbit neutrophils. J. Reticuloendothel. Soc. **30**, 167−181.

Smolen J. E., Stoehr S. J., Kuczynski B., Koh E. K. and Omann G. M. (1991), Dual effects of guanosine 5'-[gamma-thio]triphosphate on secretion by electroporated human neutrophils. Biochem. J. **279**, 657−664.

Smolen J. E. and Weissmann G. (1993), Effects of indomethacin, 5,8,11,14-eicosatetraynoic acid, and *p*-bromophenacyl bromide on lysosomal enzyme release and superoxide anion generation by human polymorphonuclear leukocytes. Biochem. Pharmacol. **29**, 533−538.

Villar A., Gascó M. A. and Alcaraz M. J. (1987), Some aspects of the inhibitory activity of hypolaetin-8-glucoside in acute inflammation. J. Pharm. Pharmacol. **39**, 502−507.

Villar A., Gascó M. A., Alcaraz M. J., Máñez S. and Cortes D. (1985), Hypolaetin-8-O-glucoside, an anti-inflammatory flavonoid from *Sideritis mugronensis.* Planta Med. **51**, 70.

Ward P. A., Sulavik M. C. and Johnson K. J. (1984), Rat neutrophil activation and effects of lipoxygenase and cyclooxygenase inhibitors. Am. J. Pathol. **116**, 223−233.

Yuli I., Tomonaga A. and Snyderman R. (1982), Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers. Proc. Natl. Acad. Sci. U.S.A. **79**, 5906−5910.

Zimmerli W., Sansano S. and Wiesenberg-Böttcher I. (1991), Influence of the anti-inflammatory compound flosulide on granulocyte function. Biochem. Pharmacol. **42**, 1913−1919.