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Anti-inflammatory effect of flavonoids from Comfort-G and the changes in arachidonic acid metabolism

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SUMMARY
Objective: Flavonoids from preparation Comfort-G have, beside of antioxidant effects, the anti-inflammatory effect, too. Comfort-G inhibits both COX-1 (cyclooxygenase-1)/COX-2 (cyclooxygenase-2) and 5-LO (5-lipoxygenase) enzymatic activity thereby decreasing the transformation of arachidonic acid (AA) to prostaglandins, thromboxanes, and leukotrienes. Our goal was to confirm this finding by the analysis of the content of AA in synovial fluid and serum of patients suffering by synovialitis of knee joint.
Design: Interventional study.
Settings: IV Department of Internal Medicine, Charles University in Prague, First Faculty of Medicine and General Teaching Hospital in Prague.
Material and Methods: Comfort-G was given to the group of ten patients (4M/6F) aged 27–79 years in prescribed dose (equal to 250 mg of flavonoid extract twice a day) for one month. Synovial fluid and serum were drawn before first application of the preparation (day 0) and one day after the last application (day 31). Free radicals (FR) were determined by direct spectrophotometric method based on chlorophyllin reaction with FR; composition of fatty acids, including arachidonic acid, was measured by gas chromatography. The probands were also asked to respond to a questionnaire (subjective evaluation) and they were also examined by the physician. Following changes of knee joint were evaluated: swelling and degree of motion-angle. Statistical analysis was performed with the help of software STATISTICA for Windows, version 4.0.
Results: The results showing the statistically significant decrease of free radical concentration in both synovial fluid and serum were presented and discussed in previously presented paper as well as the observed amelioration of patient stage. After the trial period, we found increased content of AA in both synovial fluid (4.54 ± 0.39 vs. 6.73 ± 0.82 mol%, p = 0.005) and serum (4.78 ± 0.90 vs. 6.57 ± 0.98 mol%, p = 0.01) in the same group of patients.
Conclusion: Our results confirm that flavonoids from Comfort-G are able to decrease the amount of FR and increase the concentration of AA especially in synovial fluid, but also in serum of patients. This finding corresponds with the suggestion that the enzymatic transformations of AA resulting in production of pro-inflammatory compounds are blocked. The next consequences concerning other parts of organism and impact of our results in case of other diseases are discussed.
Key words: Comfort-G, arachidonic acid, COX-2, 5-LO, inflammation.

SOUHRN
Vecka M., Prokeš L., Tvrzická E., Karpaš K., Pernický A., Pfleger R. Votruba M.: Protizánětlivý účinek flavonoidů z přípravku Comfort-G a změny metabolismu kyseliny arachidonové
Cíl práce: Flavonoidy, obsažené v preparátu Comfort-G, působí nejen jako antioxidanty, ale mají také protizánětlivý účinek. Comfort-G inhibuje nejen cyklooxygenázu-2 (COX-2), ale také 5-lipoxygenázu (5-LO), a to tím, že snižuje tvorbu prostaglandinů, tromboxanů a leukotrienů, pocházejících z metabolismu kyseliny arachidonové (AA). Naším úkolem bylo potvrdit toto tvrzení analýzou molárního zastoupení AA v synoviální tekutině a v séru pacientů, léčených pro synovialitidu kolenního kloubu.
Typ studie: Intervenční studie.
Název a sídlo pracoviště: IV. interní klinika, Univerzita Karlova v Praze, 1. lékařská fakulta a Všeobecná fakultní nemocnice v Praze
Materiál a metody: Comfort-G byl podáván skupině deseti pacientů (4 muži/6 žen) ve věku 27–79 let v předepsané dávce (odpovídající 250 mg extraktu flavonoidů užívaných dvakrát denně) po dobu jednoho měsíce. Odběr synoviální tekutiny a séra se uskutečnil jednak v den 0, tj. před podáním první dávky preparátu a v den 31., jeden den po poslední dávce. Volné radikály byly stanoveny přímou spektrofotometrickou metodou založenou na chlorofylinové reakci. Molární poměr mastných kyselin včetně arachidonové kyseliny byl stanoven plynovou chromatografií. Účastníci studie podstoupili klinické vyšetření a poskytli subjektivní hodnocení pomocí dotazníku, které se týkalo stavu kolenního kloubu. Statistická analýza byla uskutečněna softwarem STATISTICA pro Windows, verze 4.0.
Výsledky: Výsledky, ukazující statisticky významný pokles koncentrace volných radikálů, a to jak v synoviální tekutině, tak v séru, jsme prezentovali a diskutovali v předchozí studii, stejně jako zlepšení stavu pacientů. V této práci přinášíme výsledky měření obsahu kyseliny arachidonové v synoviální tekutině a séru ve stejné skupině pacientů. Po ukončení studie jsme zjistili zvýšené zastoupení AA jak v synoviální tekutině (4,54 ± 0,39 vs 6,73 ± 0,83 mol%, p = 0,005), tak v séru (4,78 ± 0,90 vs 6,57 ± 0,98 mol%, p = 0,01) ve sledované skupině pacientů.
Závěr: Naše výsledky potvrdily, že flavonoidy, obsažené v přípravku Comfort-G, jsou schopny snižovat množství volných radikálů a zvyšovat zastoupení kyseliny arachidonové, a to zejména v synoviální tekutině, ale i v séru pacientů. Toto zjištění souhlasí s tvrzením, že následující enzymatické reakce AA, které produkují prozánětlivé sloučeniny, jsou zřejmě zablokovány. Další souvislosti, týkající se jiných částí organismu, a význam našich výsledků pro jiná další onemocnění jsou předmětem diskuse.
Klíčová slova: Comfort-G, kyselina arachidonová, COX-2, 5-LO, zánět.

Introduction

Cartilage erosion in inflammatory joint diseases occurs predominantly from the lateral aspects of the joint at the junction between the invading synovium and the cartilage. Over time, there is a breakdown of the cells in joints. Age, trauma, genetic predisposition and general wear and tear stress cause the release of membrane components into joint tissue. The liberated phospholipids are then converted to arachidonic acid (AA) by phospholipase A_2 (PLA_2). Arachidonic acid can be produced in the body and plays an important role in many metabolic pathways. However, when joints are damaged, the excess of AA is converted by the COX and LO pathways into powerful inflammatory substances known as prostaglandins (PG) and leukotrienes (LT), respectively [1, 13]. While the COX pathway has been the focus of attention over the last few years, not many are aware of the LO pathway and its importance in the relief of joint pain. The LO pathway is a parallel inflammatory pathway to the COX pathway in which AA is converted to LT, the strongest chemotactic agents in the body. If left unregulated, these pathways can result in significant joint damage [5, 6].

Flavonoids are naturally occurring chemicals, which give color to plants and are found in plants, fruits, grains, nuts and vegetables. They have naturally effective anti-oxidant and anti-inflammatory properties, which can be helpful in relief of joint discomfort. Not enough flavonoids can be obtained in the diet by eating foods such as apricots, blueberries, pears, chocolate, peanuts, broccoli, white grapefruit, pomegranates, tomatoes, onions, black and green tea, red wine, parsley, kale, beans, and soybean [10, 11]. In the process of discovery and development of Comfort-G, over 1230 organic plant extracts were initially screened for COX-2 inhibitory activity. During the initial screening, 1.8% of these extracts showed inhibition of the COX-2 enzyme activity. Repeated assays confirmed that these 22 extracts remained active after re-screening. This extensive effort led to a novel product, a patent pending, proprietary blend of two classes of specific compounds, free-B-Ring Flavonoids and flavans. Comfort-G inhibits both COX-1/COX-2 and 5-LO enzymatic activity (see Table 4) thereby decreasing the metabolism of AA to PG, thromboxanes, and LT. Comfort-G also decreases the protein expression of TNF-α, IL-1β, and IL-6. Finally, this product specifically decreases key gene expression regulators, NFkB and PPARγ, which results in the down-regulation of gene expression for *cox-2*, *5-lo*, *tnfa*, *il-1b*, *il-6* but not *cox-1* [6]. Comfort-G inhibits both COX-2 and 5-LO enzymatic activity directly in the joint via interaction with the enzyme. The compound also inhibits COX-1 to the same extent but may not damage the stomach lining because it down-regulates LT production which is present in gastric ulcerations as the primary cause of tissue damage. Most potent activity arises from its ability to down-regulate the gene expression of pro-inflammatory cytokines and COX-2, which may be through the down-regulation of NFkB and PPARγ. Finally, Comfort-G acts as an antioxidant soaking up for reactive oxygen species (ROS), which can induce further production of inflammatory proteins. Comfort-G contains a high level of naturally occurring flavonoids such as baicalin and catechin (Fig. 1) that cannot be obtained in the normal European diets. The ORAC value, the measure of anti-oxidant capacity, for Comfort-G is over 5,517 UTE/g compared with vitamin C at 5,000 and vitamin E at 1000, both strong antioxidants used to supplement the diet [6].

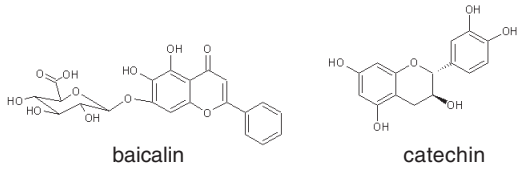

Fig. 1. Principal components of Comfort-G

Materials and Methods

Ten patients (4M/6F) aged 27–79 years, suffering from synovialitis, who have been visiting the regular controls in clinical ambulance, were selected (see Table 1 for further details). The inclusion criteria included no NSAID medication and the possibility to obtain the required amount of synovial liquid from the knee joint area twice in 30 days. Unfortunately, only these 10 persons were able to produce the required amount of synovial fluid in this time period. The other 45, which were also examined, produced only a rest of synovial fluid, which was caused by the stage of their illness and therefore they were excluded from this study.

Comfort-G was given in prescribed dose in 1 capsule (equal to 250 mg of flavonoid extract) twice a day for one month. Synovial fluid and serum intake was made on day 0 (before first application of Comfort-G) and on the day 31 (one day after the last application).

Free radicals were determined by direct spectrophotometric method based on chlorophyllin reaction with FR (Czech patent, No: 7099, kit prepared by Sevapharma a. c. Prague). Briefly, the serum samples were mixed with working solution (chlorophyllin dissolved in bicarbonate buffer with pH adjusted to 9.2 and magnesium chloride); after incubation at room temperature for 15 minutes, the absorption at 450 nm was read against the blank. The calibration solutions were based on the known concentrations of ferrous cations.

The results in Table 2 showing the statistically significant decrease of free radicals concentration in both synovial fluid and serum were presented and discussed in previously presented paper [7] as well as the observed amelioration of patient stage.

Fatty acid analysis. Total lipid was extracted from 0.5 ml of serum or 1.0 ml of synovial fluid by the method of Folch and co-workers [2] using dichlormethane instead of chloroform and trans-methylated to fatty acid methyl esters using the previously described procedure for separated cholesteryl esters [3] without hydrolysis. Gas chromatography was performed with a Shi-

Table 1. The basic characteristics of the patients

F/M	Year of birth	Diagnosis	Medication	Pain	Swell and fill retraction	Motion angle [%]	
						day 0	day 31
F	1981	Gonar III, rheumatoid arthritis, gout	Delagil ®-2r.			120*	130
F	1946	posttr.synovialitis	0			130	140
M	1968	Gonar II + synovialitis	0	receded	yes	140	140
F	1927	Gonar III	Betalog ®-2r.			100	140
M	1955	Synovialitis	0	receded	yes	140	140
F	1941	Gonar III-IV	Oxyphyllin, Nitropelet, Euthyrox-2r.			90	100
M	1930	Gonar III	Euthyrox-2r.	receded		110	110
F	1933	Gonar III	0	receded	yes	100	140
F	1931	Gonar III	0	receded	yes	100	110
M	1934	Gonar III-IV	0	receded	yes	110	120

Gonar – gonarthrosis, *physiological active motility of knee joint is 140 degrees, passively as far as to 160 degrees

Table 2. Concentrations of free radicals in body fluids after the administration of Comfort G

FA	Serum			Synovial fluid		
	day 0	day 31	P*	day 0	day 31	P*
Free radicals (mmol/l)	3.64 ± 0.32	3.44 ± 0.40[a]	NS	5.62 ± 0.30	4.82 ± 0.48[b]	0.010

[a]upper physiological limit is 6 mmol/l; [b]upper physiological limit is 1 mmol/l;*Wilcoxon paired test

Table 3. The profile of fatty acids in total lipid of serum and synovial fluid

FA	Serum			Synovial fluid		
	day 0	day 31	P*	day 0	day 31	P
14:0	0.96 ± 0.32	0.84 ± 0.28	0.2626	0.97 ± 0.26	1.03 ± 0.33	0.5751
16:0	24.79 ± 1.28	22.66 ± 1.49	0.0500	24.31 ± 1.79	22.47 ± 0.79	0.0093
16:1n-9	0.46 ± 0.06	0.67 ± 0.25	0.0929	0.45 ± 0.11	0.64 ± 0.26	0.0367
16:1n-7	2.26 ± 0.78	2.04 ± 0.62	0.3270	1.99 ± 0.65	1.75 ± 0.37	0.0593
18:0	7.69 ± 0.30	7.80 ± 0.59	0.7794	7.20 ± 0.62	7.58 ± 0.64	0.3329
18:1n-9	23.80 ± 3.67	21.37 ± 3.30	0.0687	22.41 ± 2.60	20.40 ± 1.68	0.0093
18:1n-7	1.88 ± 0.25	1.75 ± 0.26	0.1235	1.82 ± 0.31	1.67 ± 0.22	0.0593
18:2n-6	29.31 ± 5.44	31.23 ± 5.80	0.4008	32.32 ± 3.55	32.68 ± 3.22	0.7213
18:3n-6	0.33 ± 0.16	0.39 ± 0.15	0.0117	0.28 ± 0.12	0.30 ± 0.09	0.2845
18:3n-3	0.41 ± 0.12	0.43 ± 0.09	0.8886	0.44 ± 0.09	0.45 ± 0.11	0.3862
20:1n-9	0.18 ± 0.05	0.24 ± 0.09	0.4008	0.17 ± 0.08	0.18 ± 0.08	0.6465
20:3n-6	1.32 ± 0.27	1.45 ± 0.30	0.1235	1.25 ± 0.29	1.35 ± 0.26	0.0125
20:4n-6	4.78 ± 0.90	6.57 ± 0.98	0.0117	4.54 ± 0.39	6.73 ± 0.82	0.0051
20:5n-3	0.30 ± 0.10	0.45 ± 0.21	0.0357	0.36 ± 0.29	0.44 ± 0.26	0.0166
22:4n-6	0.12 ± 0.03	0.24 ± 0.07	0.0117	0.12 ± 0.02	0.27 ± 0.07	0.0051
22:5n-3	0.25 ± 0.07	0.35 ± 0.07	0.0117	0.25 ± 0.06	0.39 ± 0.07	0.0051
22:6n-3	0.77 ± 0.29	1.02 ± 0.23	0.0687	0.75 ± 0.24	1.09 ± 0.30	0.0051
Σsatur	33.56 ± 1.39	31.46 ± 2.03	0.0687	32.60 ± 1.73	31.32 ± 1.47	0.0469
Σmono	28.62 ± 4.26	26.13 ± 4.16	0.2076	26.89 ± 2.92	24.70 ± 1.83	0.0093
Σn-6	36.09 ± 5.23	40.16 ± 5.24	0.0687	38.71 ± 3.80	41.61 ± 2.69	0.0218
Σn-3	1.72 ± 0.48	2.25 ± 0.47	0.0687	1.80 ± 0.56	2.37 ± 0.61	0.0051

*Wilcoxon paired test, the data are in average ± SD format (mol%). Only the major fatty acids are listed, Σ - the sum, satur – saturated, mono – monounsaturated fatty acids, n-6(n-3) – polyunsaturated fatty acids of the n-6(n-3) family.

madzu GC-17 (Shimadzu Corp., Kyoto, Japan) gas chromatograph equipped with a capillary split/split-less injector and flame-ionization detector, combined with AOC-20S auto-sampler (Shimadzu). Analyses of FAME were performed on the fused-silica capillary columns coated with chemically bonded stationary phase DB-WAXETR (30 m x 0.32 mm I.D.) (J&W Scientific, USA). The oven temperature was programmed from 80 °C to 120 °C at 10°/min, to 200 °C at 2 °C/min, to 250 °C at 20 °/min, then isothermal 25 min. The injector and detector temperatures were 250 and 270 °C, respectively. Hydrogen carrier gas was maintained at a head pressure of 70 kPa and total flow 25 ml/min. Integration software Clarity for Windows® (Data Apex® Ltd., Prague was used for data acquisition and handling. Statistical analyses were performed with the statistical software STATISTICA for Windows, v.4.0 (Stat Soft, Inc., Tulsa, U.S.A.) [4].

Results

Patients, selected into present group, were normolipidemic persons and especially their triacylglycerol values were below of significant border 1.3 mmol/l, with regard to findings of Hjelte [8]. Because of direct and simple communication between synovial fluid and serum, one can assume the same value of AA in synovial fluid, too. This could be also inferred from the Table 3, which presents similar content of not only AA, but also that of other polyunsaturated fatty acids (PUFA) in serum and synovial fluid.

The following Table 4 gives the overview about the ability of selected NSAID drugs to inhibit both COX-2 and 5-LO reaction with relation to the effect of Comfort-G.

Discussion

The increased values of AA both in serum and especially in synovial fluid are the result of competitive inhibition of consecutive steps of AA pathway, connected with PG or LT production. There is a specificity of this model that AA is not only substrate for COX-2 and 5-LO, but in the same time the product of another enzymatic reaction too. In the same time, phospholipase A_2 also liberates the AA from membrane phospholipids. This reaction is inhibited by surplus of AA, according to the principle of feedback inhibition made by end products of the pathway [9].

The Table 3 shows that the administration of Comfort-G resulted in overall increase in PUFA (both AA and eicosapentaenoic as well as docosahexaenoic acids). These compounds are therefore not used to formation of eicosanoids (the synovial fluid as the tissue damaged should be more prone to eicosanoid formation) or the nonenzymatical degradation of PUFA is not enhanced. The level of free radicals in Table 2 indicates that the fall in free radical concentration was observed only in synovium. The third possible way for the body to get rid of PUFA molecules, β-oxidation pathway, is for these processes of minor pathophysiological importance and we can assume no disturbances in it.

As the probands did not change their dietary habits during the study, it is probable that the dietary in take of both AA and its biosynthetic precursor, linoleic acid, was not altered.

Navarro [17] published lower content of PUFA n-3 and decreased activity of delta5 desaturase, the activity of which is the precedent biosynthetic step of AA biosynthesis, in patients with rheumatoid arthritis compared to healthy controls. Moreover, the medication with NSAID did not alter the FA profile. In our group of patients, we have found higher content of PUFA n-3 and higher activity of delta5 desaturase after the Comfort-G period, the both changes indicating the shift back to physiological values.

Table 4. NSAID drugs and Comfort G inhibition of eicosanoid forming enzymes

Compound name	IC50 (COX-1)	IC50 (COX-2)	IC50 (LO)
Celecoxib	1.13	0.04	/
Refecoxib	1.9	0.5	/
Indomethacin	0.028	1.68	/
Flurbiprofen	0.29	2.56	/
Ibuprofen	1.03	14.5	/
Aspirin	1.67	278	/
Nexrutine™	0.56	/	/
Licofelone	0.16–0.21	0.18–0.23	0.18–0.23
PGV20229	7	0.22	8
Chrysin	5	5	18
Quercetin	16	16	3.5
Baicalin	0.99	0.67	9.5
Catechin	0.38	1.45	4.8
Comfort-G*	0.45	1.02	3.8
Green Tea Extract*	0.18	1.56	/

IC50 – the concentration of inhibitor (in μmol/l) reducing activity of the enzyme by 50%, / – no activity. Adapted from [18]. *The data were calculated on the basis of the average molar content of active compounds.

The Table 4 shows that only the inhibitors from the lower part of the table are able to inhibit 5-LO activity. This finding presents the fundamental point of view by choice between NSAID drugs and natural products as Comfort-G. Very successful and general inhibition, presented in Table 4, is supported by the discovery of inventor and producer of Comfort-G [6]. The preparation can influence the COX-2 production in human metabolism by the regulation of gene expression via mRNAs. Comfort-G also decreases the protein expression of TNFα, IL-1β, and IL-6. Finally, this product specifically decreases key gene expression regulators, NFkB and PPARγ, which results in the down-regulation of expression for *cox-2*, *5-lo*, *tnfa*, *il-1b*, *il-6* but not *cox-1* genes. This effect seems to be common in other groups

of dietary isoflavones. Cassidy [10] described that potent anti-atherogenic effects of these compounds include a reduction of LDL-cholesterol, modulation of pro-inflammatory cytokines, cell adhesion proteins, NO formation, protection of LDL against oxidation, inhibition of platelet aggregation and an improvement in vascular reactivity. Thunder [11] further supported these results with data on antihypertensive and antihypertrophic effect of Quercetin *in vivo* in the absence of changes concerning vascular and myocardial function. Additionally, it was found that expression of cardiac β-myosin heavy chain mRNA was reduced if Quercetin was present.

These suggestions open the new area of evaluation of our results. The inflammation proceeds by the same steps in joint, as well as in vascular wall in inflammatory phase of atherosclerosis [12]. Phospholipase A_2 has been postulated to play an important role in the inflammatory of atherosclerosis and PLA_2 could therefore be a part of crosstalk between inflammation and fibrosis, latter being an endpoint of chronic inflammation [13]. Bochkov [14] found that oxidized 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (OxPAPC) stimulated the formation of sprouts from endothelial cell spheroids. OxPAPC upregulated COX-2 and interleukin IL-8. COX-2 inhibitors as well as blocking antibodies to IL-8 suppressed activation of sprouting by OxPAPC. Finally, the authors suggested that accumulation of oxidized phospholipids may contribute to increased growth of blood capillaries in advanced lesions, thus leading to progression and destabilization of atherosclerotic plaques. A very interesting view of the necessity of some COX-2 and AA equilibrium brings the paper of Upmacis [15]. Authors determined that peroxynitrite (ONOO-) has multiple effects on COX activity. Alternatively, in the absence of AA, peroxynitrite can modify COX through nitration. In this regard, authors have shown that COX nitration occurs in human atherosclerotic tissue.

While the COX pathway has been the focus of attention over the last few years, not many are aware of the LO pathway and its importance in the relief of joint pain. The LO pathway is a parallel inflammatory pathway to the COX pathway in which AA is converted into LT, the strongest chemotactic agents in the body that cause the accumulation of cells and fluid in damaged joints. Leukotrienes, however, are a group of pro-inflammatory lipid mediators that are implicated in the pathogenesis and progression in atherosclerosis. Human lesion tissue converts AA in leukotriene B_4 (LTB_4) and the mRNA levels for the three key proteins in LTB_4 biosynthesis are significantly increased in human atherosclerotic plaque [16].

Conclusions

Comfort-G inhibits both COX-2 and 5-LO enzymatic activity directly in the joint via interaction with the enzyme. The compound also inhibits COX-1 to the same extent but may not damage the stomach lining because it down-regulates LT production which is present in gastric ulcerations as the primary cause of tissue damage. The inhibition of all major eicosanoid pathways can be derived from the increased content of AA in lipids. Finally, Comfort-G acts as an antioxidant. Based on the universal effect on all steps of inflammation, Comfort-G could be used as an anti-inflammatory preparation not only in case of a joint disease, but in case of all forms of inflammation, especially in atherosclerosis. If the drugs acting as specific inhibitors of COX-2 have to be eliminated from pharmacologic practice, because of their support of atherosclerotic process, the flavonoids from Comfort-G help not only in case of diseases of locomotor system but in case of atherosclerosis developing, too.

References

1. **Namgaldaze, D., Bruene, B.** Phospholipase A2 – modified LDL activates the phosphatidylinositol3-kinase-akt pathway and increases cell survival in monocyte cells, *Arterioscl. Thromb. Vasc. Biol.,* 2006, 26, 11, p. 2510–2516.
2. **Folch, J., Lees, M., Sloane-Stanley, G. H.** A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* 1957, 226, 1, p. 497–509.
3. **Vecka, M., Kunešová, M., Staňková, B. et al.** Změny složení mastných kyselin v séru a tukové tkáni u obézních žen po velmi přísné nízkoenergetické dietě. *Klin. Biochem. Metab.* 2001, 12, 33, 3, p. 119–124.
4. **Tvrzická, E., Vecka, M., Staňková, B., Žák, A.** Analysis of fatty acids in plasma lipoproteins by gas chromatography--flame ionization detection. Quantitative aspects. *Anal. Chim. Acta,* 2002, 465, p. 337–350.
5. **Xie, W. L., Robertson, D. L., Simmons, D. L.** Mitogen-inducible prostaglandin G/H synthase – a new target for nonsteroidal antiinflammatory drugs. *Drug Dev. Res.,*1992, 25, 4, p. 249–265.
6. **Jia, Qi, Nichols, T. C., Rhoden, E. E. et al.** Unigen Pharmaceuticals, Inc.: Identification of free-B-ring flavonoides as potent COX-2 inhibitors, U.S. Patent no. 7192611, 2003.
7. **Prokeš, L., Karpaš, L., Pernický, A., Pfleger, R., Votruba, M.** *Analgetický a protizánětlivý účinek flavonoidů (Comfort-G)* In Sborník *Nejčastější onemocnění ve stáří, strategie v terapii, diagnostice a následné péči.* KREPRO : Hradec Králové 2006, s. 67.
8. **Hjelte, L. E., Nilsson, A.** Arachidonic acid and ischemic heart disease. *J. Nutr.,* 2005, 135, p. 2271–2273.
9. **Devlin, T. M.** *Textbook of biochemistry with clinical correlations.* 3rd edition, U.S.A., Wiley-Liss : New York 1992, p. 188–189, ISBN 0-471-51348-2.
10. **Cassidy, A., De Pascual-Teresa, S.** *Dietary isoflavones and coronary artery disease-proposed molecular mechanisms of action.* In Rimbach, G. et al. *Nutrigenomics.* CRC Press : London 2005, p. 301–326.
11. **Thunder, J., Carlstrom, J., Sun, K. et al.** Quercetin--supplemented diets lower blood pressure and attenuate cardiac hypertrophy in rats with aortic constriction. *J. Cardiovasc. Pharmacol.,* 2006, 47, 4, p. 531–541.
12. **Calabro, P., Yeh, E. T. H.** *Inflammatory vascular markers in atherosclerosis.* In Geng, Y. J. *Current Topics in Atherosclerosis Research.* Nova Science Publishers, Inc. : New York, U.S.A. 2005, p. 49–65.

13. **Oestvang, J., Johansen, B.** Phospholipase A_2: A key regulator of inflammatory signalling and a corrector to fibrosis development in atherosclerosis. *Biochem. Biophys. Acta, Molec. Cell. Biol. Lipids,* 2006, 176, 11, p. 1309–1316.
14. **Bochkov, V. N., Philippova, M., Oskolkova, O. et al.** Oxidized phospholipids stimulate angiogenesis via autocrine mechanisms, implicating a novel role for lipid oxidation in evolution of atherosclerotic lesions. *Circulation Res.,* 2006, 99, 8, p. 900–908.
15. **Upmacis, R. K., Deeb, R. S., Hajjar, D. P.** Oxidative alternations of cyclooxygenase during atherogenesis. *Prostaglandins Other Lipid Mediat.,* 2006, 80, 1–2, p. 1–14.
16. **Qiu, H., Gabrielsen, A., Agardh, H. E. et al.** Expression of 5-lipoxygenase and leukotriene A_4 hydrolase in human atherosclerotic lesions correlates with symptoms of plaque instability. *Proc. Nat. Acad. Sci. U.S.A.,* 2006, 103, 21, p. 8161–8166.
17. **Navarro, E, Esteve, M., Olivé, A. et al.** Abnormal fatty acid pattern in rheumatoid arthritis. A rationale for treatment with marine and botanical lipids. *J. Rheumatol.,* 2000, 27, 2, s. 298–303.
18. **Unigen scientists** *Table of Content Concerning the Scientific Aspects of Information Necessary to Support Applications for the Placing on the Market of Novel Foods and Novel Food Ingredients, confidential paper.* U.S.A., 2007.

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