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Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat

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Abstract

The anti-inflammatory activities of three flavonoids were investigated in rats using the Mizushima et al. model of acute and chronic inflammation. Intraperitoneal administration of rutin, quercetin (flavonols) and hesperidin (flavanone), given at daily doses equivalent to 80 mg/kg, inhibited both acute and chronic phases of this experimental model of inflammation. Rutin was the most active in the chronic phase.

Keywords: Flavonoids; Rutin; Quercetin; Hesperidin; Anti-inflammatory activity; Adjuvant arthritis

1. Introduction

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described [1], and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. Flavonoids have a variety of biological effects in numerous mammalian cell systems, in vitro as well as in vivo. They have been shown to exert antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, anti-inflammatory, antioxidant, antihepatotoxic, antihipertensive, hypolipidemic and antiplatelet activities [2]. Flavonoids were investigated in models of inflammation in rats and were found to possess significant activity in both proliferative and exudative phases of inflammation [3]. Studies on the in vivo anti-inflammatory activity of flavonoids showed that inhibition of both paw edema and edema was caused by croton oil [4]. We have previously reported that flavonoids showed anti-inflammatory activity and inhibited the development of the induced granuloma, mostly when a catechol or guaiacol-like B ring is contained in the compound structure [5].

Some flavonoids, such as quercetin, blocked both the cyclooxygenase and lipooxygenase pathways at relatively high concentrations, while at lower concentrations, the lipooxygenase pathway was the primary target of inhibitory anti-inflammatory activity [6]. A micronized flavonoid complex, consisting of 90% diosmin + 10% hesperidin (Daflon 500 mg), protected against the formation of perivascular edema and its therapeutic values were determined by its inhibitory activity on the inflammatory process [7]. On the other hand, when administered subcutaneously, hesperidin (hesperetin-7-rutinoside), although inactive *per os*, exhibited significant anti-inflammatory activity on rat paw edema induced by both carrageenan and dextran and on carrageenan pleurisy, without producing the side effects that are caused by other classes of anti-inflammatory drugs [8].

Some authors have reported that flavonoids such as rutin (quercetin-3-rutinoside) and quercetin show antioxidant activity [9,10]. In rheumatoid arthritis, acute and chronic inflammation occur together in synovial

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pannus. Features of chronic inflammation, inducing altered expression of matrix metalloproteinases and formation of new blood vessels, are stimulated by prostaglandins [11]. In the regulation of prostaglandin production, cyclooxygenase is an enzyme that represents the critical control point [12]. This enzyme is a target of pharmacologic action of the non-steroidal anti-inflammatory drugs.

The pharmacological treatment continues to be inadequate in preventing the progression of this disease to the stage of irreversible joint erosion and deformity.

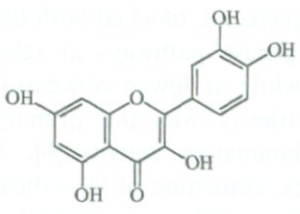
The development of new anti-inflammatory drugs continues to be essential not only because of the need for compounds that will prevent joint destruction but also because compounds with unique mechanism of action can be used as tools to further clarify the pathogenesis of rheumatoid arthritis [13].

There is growing interest in the pharmacological potential of natural products and the purpose of our study, therefore, was to investigate the ability of quercetin, rutin and hesperidin to inhibit acute and chronic inflammation on experimental arthritis. Schemes 1 and 2

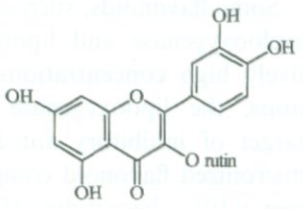
2. Experimental

2.1. Animals

Experimental animals: female Wistar rats, weighing 150–180 g, were used in these experiments. They were randomly assigned to different groups and a period of 4 days was allowed for adaptation. Animals were provided with food (standard chow diet manufacturer) and water *ad libitum* and were maintained at a constant temperature of 22 ± 1°C and humidity of 55 ± 5%.

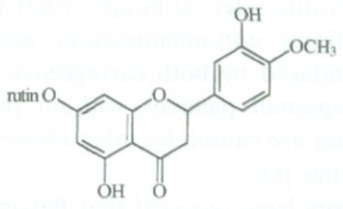


quercetin



rutin

Scheme 1.



hesperidin

Scheme 2.

2.2. Anti-inflammatory evaluation

Experimental arthritis was induced in rats according to the method of adjuvant-carrageenan-induced inflammation (ACII) [14]. A total of 35 rats was divided into control, standard and test groups of seven animals each. On day 0, all of them received an injection of 0.1 ml Freund's complete adjuvant (Difco; Sigma, USA) intradermally at the base of the tail. After six days of adjuvant inoculation (day 6), a suspension of 0.1 ml of carrageenan (type IV; 2% w/v in saline solution) (Sigma, USA) was injected in the subplantar region of the left hind paw of the rat, 1 h after the intraperitoneal administration of rutin, quercetin, hesperedin and phenylbutazone. The degree of pedal edema was determined by measuring the volume of both hind paws by plethysmography (Ugo Basile). Plethysmographic measurements were made before the adjuvant injection (day 0), and were repeated again 6 days later at 3, 5 h (acute phase) and discontinued from days 7 to 30 (chronic phase) after carrageenan injection.

Rutin, quercetin (test groups) in doses of 80 mg/kg and phenylbutazone (standard group) 80 mg/kg (Sigma, USA) were administered intraperitoneally 1 h before the carrageenan injection and once every day at the same time throughout the experiment; hesperidin (test group) was administered only until day 21. For the control group, the vehicle used was saline solution. The inhibition percentage of edema was calculated for each animal group in comparison with the control group.

Arthrogram scores [15] were obtained on days 21 and 30 by visually grading the following on 0 to 2+ basis (0, none; 1+, moderate; 2+, severe): hind paws, ankylosis; ears redness and/or nodular lesions; and tail, ankylosis and/or nodules.

2.3. Statistical analysis

Data obtained from the pharmacological experiment are expressed as mean ± SEM. Differences between the control and the treatments in this experiment were tested for significance using Dunnet's *t*-test [16]. A probability of $P < 0.05$ was considered significant and a probability of $P < 0.01$ was considered very significant.

3. Results

The effect of flavonols (rutin and quercetin) and flavanone (hesperidin) are shown in Table 1.

There was no significant difference in paw edema between basal measure (day 0) prior to adjuvant and that just prior to the carrageenan injection (day 6, 0 h).