ISSN: 2348-8948
Vol: 2; Issue: 5

RESEARCH ARTICLE

ANTI-INFLAMMATORY ACTIVITYOF QUERCETININ ANIMAL MODELS OF CHRONIC INFLAMMATION

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Date Received:	Date of Accepted:	Date Published:
26thApr2014	4thMay2014	12thMay2014

Abstract:
The side effects of the currently used anti-inflammatory agents are the major problem during their clinical use; therefore, development of newer, more effective and safe anti-inflammatory drugs is necessary. The present study evaluates the anti-inflammatory activity of quercetin in animal models of chronic and granulomatous inflammation.Forty-eight rats were used to induce chronic inflammation in the hind paw with formalin and granulomatous inflammation with sterile cotton-wool pellets. The anti-inflammatory activity of quercetin (150 mg/kg, P.O) was evaluated in the two models, and compared with that produced by dexamethasone (1 mg/kg, P.O). Quercetin decreased significantly the formation of exudate and granulation tissue compared with the vehicle, but still significantly lower than that produced by dexamethasone. In conclusion, quercetin inhibits the production of edema and granulation tissue in animal models of chronic and granulomatous inflammation;it could be a potential choice for the treatment of chronic inflammatory disorders.

Keywords: quercetin, chronic inflammation, granuloma, rats

Introduction

Inflammation is an important physiological reaction, which occurs in response to a wide variety of injurious agents (bacterial infection or physical trauma) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair [1]. It requires the participation of various cell types expressing and reacting to diverse mediators along a very precise sequence [2]. Although inflammation is beneficial in the setting of defense of the host against infectious invaders, it may become unchecked contributing to the pathogenesis of common chronic inflammatory diseases such as atherosclerosis, obesity induced insulin resistance, arthritis, inflammatory bowel disease and multiple sclerosis [3,4]. Chronic inflammation begins 2-4 days after the onset of acute response and can last for weeks to months or years due to the persistence of the initiating stimulus, interference of the normal healing process, repeated bouts of acute inflammation or low-grade smoldering due to continued production of immune response mediators [5]. Attenuation of the chronic inflammatory response is a beneficial strategy to combat several human diseases. Although steroidal and non-steroidal anti-inflammatory drugs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders because such compounds are accompanied by unexpected side effects [6,7]. Therefore, there is an urgent need to find safer anti-inflammatory compounds, and traditional medicine offers many plant extracts and pure natural compounds as treatment options of a wide variety of disorders including acute and chronic inflammation [8]. Among the natural active constituents, quercetin is a phytochemicalthat belongs to the flavonoids class. Flavonoids are compounds found in fruits and vegetables; to date, more than 4,000 have been identified. The flavonoids have generated scientific interest because of their potential beneficial effects on human health, including antioxidant,

anti-inflammatory, antitumor, and antiviral activities. Quercetin, the major flavonoid in the human diet, is widely found in apple skins, onions, berries, grains, herbs, tea, and red wine [9]. It can inhibit nuclear factor-kappa B (NF-κB), which plays a central role in regulating the immune response to inflammation. Cell culture and whole animal studies have provided evidence supporting the anti-inflammatory effects of quercetin [10]. Recently, some studies have also reported that, in vitro, quercetin can inhibit various cytokines, including tumor necrosis factor-α (TNF-α) [11,12].Therefore, the present study was designed to evaluate the anti-inflammatory effect of quercetin in experimental animal models of chronic and granulomatous inflammations.

MATERIALSAND METHODS

Forty eight 10 weeks old, male Sprague-Dawley rats were housed in the animal house, School of Pharmacy, University of Sulaimani in well ventilated plastic cages, at an ambient temperature (25±2°C) and humidity (55±5%) under 12hr dark-light cycle. Experimental protocols met the guidelines for animal experimentation and approved by the Ethical Committee of the Faculty of Medical Sciences, University of Sulaimani. The animals were randomly allocated in to 2 groups, according to the type of inflammatory model, of twenty-four rats in each group.

Formalin-Induced Chronic Inflammation:

In this model, chronic inflammation was induced by injecting 0.1ml of 2% formaldehyde into the sub-planter area of the right hind paw of ether-anesthetized rat. Both drugs including quercetin (150 mg/kg body weight), which is formulated as suspension in 5% cabroxy-methylcellulose (CMC) in distilled water, as tested drug. The selected dose of quercetin was determined to be safe based on previous studies [13]; dexamethasone (1 mg/kg body weight) as a standard drug, and the vehicle distilled water (0.2 ml/100g body weight), given to the control group. All were given 30 min prior to formaldehyde injection, and continued for seven consecutive days. Both drugs and the vehicle were given orally as once daily doses. In this model, the increase in paw thickness was measuredutilizing the vernier caliper method. The paw thickness was measured before and 6 days after induction of inflammation, and presented as mean increase in paw thickness (mm) [14]. The ability of the drugs to suppress paw inflammation was expressed as a percentage of inhibition of paw edema and this percentage can be calculated according to the following equation [15]:

Percentage of inhibition (%) = (C – T) / C x 100

Where C = increase in paw thickness of control group of rats and, T = increase in paw thickness of treated group of rats.

Cotton Pellet-Induced Granulomatous Inflammation:

The cotton pellets-induced granuloma was performed using the method of Winter and Porter [16]. Cotton pellets weighing 10±1 mg were sterilized in an autoclave for 30 min at 120°C under 15 Lb pressure. Four pellets were implanted subcutaneously (S.C.), into the ventral region, two on either side, in each rat under light ether anesthesia. Both drugs (quercetin 150 mg/kg and dexamethasone 1 mg/kg) and the vehicle (distilled water 0.2 ml/100 gm) were given orally for seven consecutive days from the day of cotton pellet implantation. On 8th day, the animals were anesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed for wet weight and then dried in an incubator at 60°C for 18 hr until a constant weight was obtained (all the exudates was dried); after that the dried pellets were weighed again [17]. The exudate amount (mg) was calculated by subtracting the constant dry weight of pellet from the immediate wet weight of pellet. The granulation tissue formation (dry weight) was calculated after deducting the weight of cotton pellet (10 mg) from the constant dry weight of pellet; and considered as a measure of granuloma tissue formation. The percent inhibitions of exudate and granuloma tissue formation were determined as follows:

Exudate inhibition (%) = {1 – Exudate in treated group / Exudate in controls} x 100.

Granuloma inhibition (%) = {1 – granuloma in treated group / granuloma in controls} x 100.

Statistical Analysis

All the results were expressed as mean±SEM. Analyses of data was performed utilizing Graph Pad Prism software for Windows (version 5.0, Graph Pad Software, Inc., San Diego, CA). The significance of differences among the studied groups was determined using one-way analysis of variance (ANOVA) and Bonferroni's*post hoc* analyses. Values with $P<0.05$ were considered significant.

RESULTS

In Table 1, quercetin (150 mg/kg body weight) significantly reduced the increases in paw thickness compared to controls, with maximum inhibitory effect on edema formation (92.4%) after 7 days of challenge with formalin. Meanwhile, 1 mg/kg dexamethasone

significantly inhibited the increases in paw thickness compared to controls (71.8%). The effects of quercetin and the standard drug (dexamethasone) were found comparable, and no significant differences reported when all the results analyzed using ANOVA. Table 2 shows that orally administered quercetin (150mg/kg) did not significantly decrease the formation of inflammatory exudate compared to controls, with inhibition level of exudate formation around 19.1% (P<0.05).

Meanwhile, 1 mg/kg of dexamethasone significantly inhibited exudate formation compared to controls (59.2%), and significantly higher than that produced by quercetin (P<0.05). Quercetin also did not significantly decrease the formation of granuloma compared to control (P>0.05), with inhibition level of granuloma formation around 16.7% (P>0.05). Meanwhile, 1 mg/kg dexamethasone significantly inhibited the formation of granuloma compared to controls (69.8%); this effect was significantly higher than that produced by quercetin (Table 2).
Figure 1 indicates that the anti-inflammatory activity of quercetin, in terms of inhibition of paw thickness, was poorly and non-significantly correlated with that reported in the model of cotton wool-induced granuloma; where quercetin demonstrates weak anti-inflammatory activity in the later model. In contrast to that, figure 2 shows good correlation between the anti-inflammatory activities of dexamethasone in the two models, and they are non-significantly different.

DISCUSSION

Dietary polyphenols comprise a vast array of biologically active compounds that are ubiquitous in plants, many of which have been used in traditional Oriental medicine for thousands of years [8]. Flavonoids are abundantly present in fruits, vegetables, seeds, nuts, tea, and red wine, and the flavonoid mostly consumed is quercetin. Flavonoids are believed to act as health-promoting substances, and some of them have antioxidant and anti-inflammatory properties [18,19]. Formalin induced paw edema is one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembled human arthritis [20]. In the present study, oral administration of Quercetin (100mg /Kg body weight) produced significant reduction in paw thickness and results in 92.4 % inhibition in paw edema; it also results in 16.7% inhibition of granuloma formation. This finding was in tune with those reported by others [21,22]. In the present study, we have evaluated the anti-inflammatory role of quercetin in animal models of chronic and granulomatous inflammation demonstrating that this polyphenol attenuated formalin- and cotton wool-induced inflammatory events revealed as reduction in the degree of edema, exudate and granuloma formation a range of clinical conditions are associated with a dysregulation of inflammatory responses. In one study, treatment with quercetin reduces the serum level of CRP in animal models of low-grade inflammation that predispose to diabetes [23]. Furthermore, other previous studies demonstrated that quercetin decreases the elevated serum level of CRP during progression and regression of atherosclerosis using hypercholesterolemic dietin rabbits [24]. Quercetin also decreases the expression of endogenous TNF-α gene [11]. Quercetin also affects the upstream signaling of NF-κB pathway by inhibiting up regulation of members of the IKK complex, and these effects on the IKK cascade would in turn contribute to inhibition of NF-κB activation [25]. In addition, the effect of quercetin on NF-κB may be mediated through decreasing the phosphorylation state of IκBa and IκBb which provided a direct mechanism by which quercetin can inhibit the activity of NF-κB, therefore decreasing endogenous TNF-α expression [11] and down-regulating CRP expression [26]. In animal model of rheumatoid arthritis, quercetin inhibits the activity of matrix metalloproteinase-2 (MMP-2) [27], thereby inhibiting the degradation of basement membrane, and indirectly inhibiting angiogenesis and cartilage damage. *In vivo* animal experiments also support an anti-inflammatory effect. Quercetin ameliorates the inflammatory response induced by carrageenan [28], and a high-fat diet [29]. Quercetin reduced visceral adipose tissue TNF-α and nitric oxide production and down-regulated NOS expression in obese Zucker rats [30]. In chronic rat adjuvant-induced arthritis, quercetin decreased clinical signs of arthritis compared to untreated controls [31]. Thus, the anti-inflammatory activity of quercetin administration in these models might be due to direct effect of quercetin on many mediators of inflammation at the used dose and duration of administration. The results of the present study are in tune with those observed with silibinin, another potent flavonoid that shows remarkable anti-inflammatory activity in animal models of acute and chronic inflammations [32]. All these including the present study, proved that several flavonoids including silibinin really inhibit the expression of pro-inflammatory molecules [33] in experimental animal models and human studies. These findings suggested that modulation of pro-inflammatory mechanisms is certainly one of the major actions, or mechanisms of flavonoids that may explain their anti-inflammatory activity. In conclusion, quercetin shows remarkable anti-inflammatory activity in animal models of chronic and granulomatous inflammation.

ACKNOWLEDGEMENT

The authors thank University of Sulaimani for supporting the project, and the School of Pharmacy for technical assistance.

Table 1.Anti-inflammatory activity of quercetin (150mg/kg, P.O) in animal model of formalin-induced chronic inflammation.

Treatment group	Mean paw thickness at baseline (mm)	Mean paw thickness after 7 days (mm)	Mean changes in paw thickness (mm)	Inhibition of paw edema (%)
Control	3.8±0.2	7.2±0.42*[a]	3.4±0.44[a]	8.42±13.6[a]
Dexamethasone 1mg/kg	3.7±0.11	4.63±0.22*[b]	0.96±0.2[b]	71.8±7.2[b]
Quercetin 100mg/kg	3.5±0.15	3.7±0.18*[b]	0.22±0.08[b]	92.4±3.03[b]

Values are presented as mean±SEM; n=6 rats in each group; values with non-identical superscripts (a,b,c) among different groups considered significantly different (P<0.05).

Table 2.Anti-inflammatory activity of quercetin (150mg/kg, P.O) in animal model of cotton wool-induced granuloma.

Treatment group	Weight of exudate (mg)	Inhibition of exudate %	Weight of granuloma (mg)	Inhibition of granuloma %
Control	173.8±14.7[a]	-	39.2±3.3[a]	-
Dexamethasone 1mg/kg	69.2±2.6[b]	59.2±2.6[a]	11.7±1.0[b]	69.8±1.9[a]
Quercetin100mg/kg	139.7±7.3[a]	19.1±7.4[b]	33.0±3.5[a]	16.7±2.9[b]

Values are presented as mean±SEM; n=6 rats in each group; values with non-identical superscripts (a,b,c) among different groups considered significantly different (P<0.05).

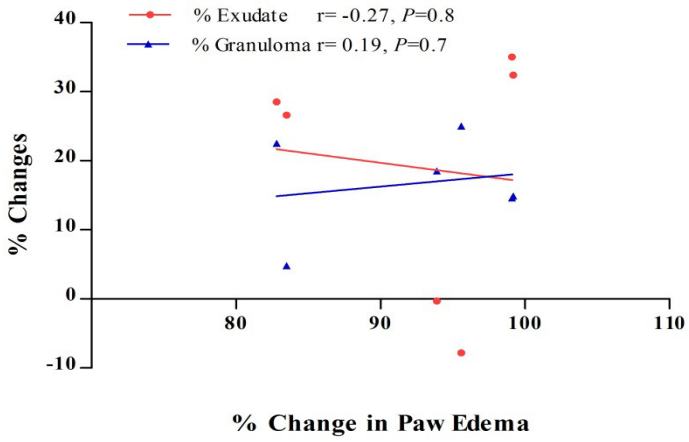

Figure 1: Correlation between the anti-inflammatory effects of quercetin (150mg/kg) in formalin-induced chronic inflammation and cotton wool-induced granuloma.

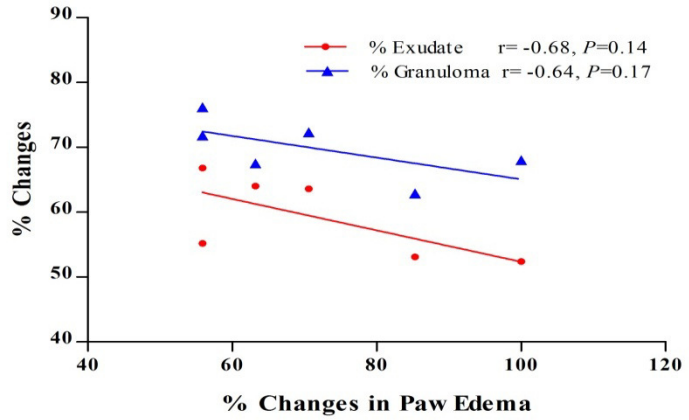

Figure 2: Correlation between the anti-inflammatory effects of dexamethasone (1.0mg/kg) in formalin-induced chronic inflammation and cotton wool-induced granuloma.

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