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Quercetin Effectively Quells Peanut-Induced Anaphylactic Reactions in the Peanut Sensitized Rats

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ABSTRACT

Peanut allergy is the major leading cause of fatal or life-threatening anaphylactic reactions to foods. At present, there is no remedy for this condition. The applied pharmaceutical cares are merely palliative, while their deleterious side effects have already been established. Hence, many sufferers search for complementary and alternative medicines. A versatile-, "flavonol" subgroup-member of the flavonoid family, quercetin, is of paramount interest to investigators. In this study the effects of quercetin on peanut-induced anaphylactic reactions were investigated in a rat model of peanut allergy. Wistar rats were sensitized with crude peanut extract in the presence of Cholera toxin and Aluminium hydroxide. Sensitized rats were then allotted into three groups; Positive control, Quercetin-treatment and Sham, (n=7, each). Naive rats (n=7) served as negative controls.

One week post-sensitization period, the rats in treatment group were treated with quercetin at a dose of 50 mg/kg(Body Weight)/mL Di-methyl-sulfoxide 5%/rat, over a period of four weeks. Subsequently, rats were challenged, and anaphylactic reaction parameters including variations in plasma histamine levels, vascular permeability, systemic anaphylaxis scores, and total serum Immunoglobulin E levels were measured.

After daily-gavaging for four weeks, quercetin completely abrogated peanut-induced anaphylactic reactions following challenges, so that the mean of plasma histamine levels in the quercetin-treated rats, were lower significantly ($p=0.004$) as compared with positive control group. Our findings suggest that the flavonoid quercetin is potent enough to suppress the on-going Immunoglobulin E responses against peanut proteins, and can be propounded as an alternative medicine to protect against Immunoglobulin E-mediated food allergies.

Key words: Complementary and Alternative Medicines; Flavonoids; Food allergy; Peanut allergy; Quercetin; Wistar rats

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INTRODUCTION

Food allergy, defined as an adverse immunological reaction to food, entails a plenty of public health complications particularly in children, due to the prospective severity of allergic hypersensitivities. Food allergic responses involve more often Immunoglobulin E (IgE)-mediated reactions[1] and failure to develop, and/or a breakdown in oral tolerance can result in the production of specific IgE antibodies against the allergenic food in contemplation. IgE and food allergen(s) activate mast cells and basophils via high affinity IgE receptors (FcεRIs). This activation triggers the release of a whole host of chemical mediators through the effector cells degranulation, among them histamine is considered as the most important mediator that can cause all the pathological features of allergic diseases.[2] IgE-dependent food-allergic reactions may engage one or more organs including the skin, the gastrointestinal tract, and the respiratory tract. In the severe cases, the cardiovascular system is also affected, followed by systemic shock expansion.

Amongst such wearisome ailments (IgE-mediated food allergies), peanut(PN) allergy is an overwhelming type representing by own itself,[3] the most frequent leading cause of fatal and life threatening anaphylactic reactions to foods.[4-6] Unfortunately, the only proven treatment consists of its all possible food/allergen-sources meticulous avoidance On the other hand, many accidental exposures (up to 50% of patients per year[7,8]) occur owing to the ubiquitous use of PN allergens in a variety of food products. Thus, given the large number of patients with potentially fatal PN allergy, of inevitable cases of accidental exposures and the lack of efficacy for standard immunotherapy, more effectual and at the same time, safe prophylactic and/or curative approaches are urgently needed.

Concerning the allergies, compounds of interest are flavonoids in general, and quercetin in particular, which are present in daily often-consumed foods in fairly high levels.

Quercetin, a natural compound belonging to "Flavonol"-subgroup of the flavonoid family, is the aglycone (non-carbohydrate portion) of rutin, quercetrin and other glycoside flavonoids. It is widely distributed in the plant Kingdome including oaktrees (Quercus spp.), onions (Allium cepa) and tea (Camellia sinensis), and is found in many human foods such as apple, onion, tea, berries, and brassica vegetables, as well as many seeds, flowers, barks, and leaves.[9] Quercetin is often a major component of the medicinal activity of the plant, and has been shown in experimental studies to have numerous effects on many different biological systems in the body; most of them are executed via its interaction with the calcium-regulating enzyme, i.e. Calmodulin.[10] It also has revealed great and wonderful inhibitory effects on histamine release in some In vitro systems.[11-15]

Because of its anti-allergic and anti-inflammatory properties, quercetin has been proposed for the prevention or therapy of allergic and chronic inflammatory diseases and might be useful for the treatment of food allergies.

In the present study we report the anti-allergic properties of quercetin by evaluating its effects on some pre-established major causative determinants of the anaphylactic reactions in a wistar model of PN allergy.

MATERIALS AND METHODS

Animals

Male Wistar rats (4-6 weeks old) weighing 70-130 g at study initiation, were purchased from the Animal Research and Care Center of Ahvaz Jondishapour University of Medical Sciences (AJUMS). Animals were housed in colony cages (7 rats per cage) in our laboratory conditions maintained at an ambient temperature of 23±3ºC with a relative humidity of 30-70 % and a light/dark cycle of 12h during the experiment and for at least one week prior to sensitization period (for acclimatization purpose). All rats had access to PN-free standard laboratory rodent chow and water ad libitum.

All procedures involving animals were conducted in accordance with the Guidelines for Laboratory Animal Experiments in AJUMS Animal Research and Care Center.

Reagents

The reagents used in the experiments, were purchased from the source shown in parentheses: Aluminium hydroxide (Alum) (Alhydrogel 2.0%, Serva Chemical Co., U.S.A), Cholera toxin (C-3012, Sigma Chemical Co., St. Louis, Mo, U.S.A), DMSO (Merck Chemical Co., Germany), Evan's blue dye (Merck Chemical Co., Germany), Rat histamine kit (LDN Chemical Co., Germany), K_3-EDTA (Sigma Chemical

Co., St. Louis, Mo, U.S.A), PBS (Merck Chemical Co., Germany), Quercetin (Q# 0125, Sigma Chemical Co., St. Louis, Mo, U.S.A), Rat Total IgE kit (ICL Chemical Co, U.S.A).

Unshelled/incrusted crude PNs were supplied from Safee-Abad Tree-plantar Research Station in the town of Dezfool.

Antigen Preparation

In this investigation, PN proteins were used as antigen which were extracted from fresh crude PNs as follows, briefly:

PN bodies were grounded by a mill and the resulted paste was defatted by n-Hexane (1:3 v/v, 3 times). Subsequent to separation process, residues were desiccated and deodorized via gentle heat-treatment. Then, the obtained flour was mixed with Phosphate Buffered Saline (PBS) (1:10 w/v) and subjected to extraction by shaking over night at 4 ºC. The resulted suspension was centrifuged twice, in order to clarification as described below:

First time: centrifugation at 3500 r/m and 4 ºC for 30 min.

Second time: centrifugation at 5000 r/m and 4 ºC for 20 min.

After the later, the supernatant was filter-sterilized through 0.45-µm pore size sterile syringe filters and the collected extract was frozen at -20 ºC until use. Preparation procedures were performed in the pharmacy faculty of AJUMS, and the quantitative operations including determination of the "protein percentage" of the extract under investigation, were done at food-industries laboratory of the faculty of nutrition sciences in the university aforementioned.

PN-sensitization/-challenges

Pre-study blood samples were tested to ensure the use of immunologically naive animals with respect to the allergen investigated. 35 out of all 50 rats, were subjected to sensitization three times, one week apart with crude PN extract (CPE) according to Roy K, et al., 1999,[16] with minor modification. Each sensitization attempt was done over 2 consecutive days (on days #8,9…#16,17…#24,25, respectively).

Day #1: intragastric (ig) administration of 1 mg CPE plus 10 µg Cholera toxin per rat.

Day #2: intraperitoneal (ip) injection of 0.5 µg CPE plus 0.2 mL Alum per rat.

The sensitized rats were then divided into 3 groups: QRS-treatment, sham and positive control (n=7, each). Non-sensitized (naive) rats (as negative controls; n=7) were studied in parallel, for comparison purposes.

Interventional Procedure

One week post-sensitization period (on day #32), the rats in treatment group received daily, quercetin powder (50mg/kg.BW) dissolved in 5% dimethyl sulfoxide (DMSO) aqueous solution (as vehicle) by interagastric gavaging, over a period of four weeks.[17] The used dose of quercetin was calculated considering a conversion table of equivalent effective dose ratios from human beings to animals, based on body surface areas (BSA). In the same manner, the rats in sham group and in both of the control groups, were gavaged with 1 ml/rat of 5% DMSO aqueous solution and tap water, respectively, throughout the intervention phase.

During treatment, the rats in all sensitized groups were orally boosted with a single dose of CPE (1 mg/rat) plus Cholera toxin (10 µg/rat) to maintain hypersensitivity (twice; 2 and 4 w after the latest sensitizing-dose administration).

Assessment of Systemic Anaphylactic Symptom/signs

Anaphylactic symptom/signs were evaluated 30-40 min after the second ig challenge dose (1 mg of CPE/rat, as the first one (but), 30 min later) by using the scoring system which was modified slightly, from the previous descriptions.[18,19]

0: no signs;
1: scratching and rubbing around the snout and head;
2: pilar erecti, puffiness around the eyes and mouth, teeth-gnawing (is reported for the first time), diarrhea, (urine) incontinence (is reported for the first time), anorexia (is reported for the first time), reduced activity and/ or standing still with increased respiratory rate;
3: wheezing, labored respiration, and cyanosis around the mouth and the tail;
4: symptoms as in no. 3 with no activity after prodding, lethargy/paralysis (is reported for the first time), or tremor and convulsions;
5: death.

Measurement of Rectal Temperature

Rectal temperatures were measured 20-25 min after the first ig challenge dose by using a digital thermal

probe apparatus, pre- and post-intervention, same as all the other accomplished differentiative tests.

Measurement of plasma histamine levels

Orbital plexus blood samples were collected by microcapillary hematocrit tube into EDTA microtubes (1.5 mL in size, 1 mL in each one/rat) for plasma analysis of histamine. Specimens were obtained 25-30 min following the second ig challenge and centrifuged at 2000×g for 20 min and plasma (supernatant) was stored at −20°C until analyzed by using an enzyme immunoassay kit, according to the manufacturer's instructions. All analyses were performed in duplicate.

Wheal Reaction

Before PN-challenge, the abdominal surface of rats (been shaved a few hours earlier; 4 rats/group) was used for intradermal (id) skin testing with sterile CPE. Five min before the test, 100 µL of Evan's blue dye (5 mg/mL PBS) was injected into the tail vein to help visualize the wheal reaction. Subsequently, 66 µL of the filter-sterilized CPE (3 mg/mL) was administered intradermally into the previously shaved abdominal skin. Negative controls (Saline) were placed as well. A positive test response was defined as a wheal reaction showing up as a blue area measuring greater than 5mm in diameter when read at 15-20 min post id PN-challenge.

Detection of Vascular Leakage

Five min before ip PN-challenge dose, 3 rats from each group received 200 µL of Evan's blue dye (5 mg/mL PBS) by tail vein injection. Footpads of mice were examined for signs of vascular leakage (visible blue color) 45 min after ip administering of 200 µg of the filter-sterilized CPE.

Measurement of Total Serum IgE Levels

Orbital plexus blood samples were collected four times, by microcapillary hematocrit tube into microtubes (1.5 mL in size, 1 mL in each one/rat), at the mid of acclimation period, one week post-sensitization period and biweekly afterwards.

After 0.5- to 1-h coagulation at room temperature, sera were collected and stored at −20°C until analyzed. Levels of total IgE immunoglobulin were determined by using an enzyme immunoassay kit, as described by the manufacturer. All analyses were performed in duplicate. At one week post-sensitization period, the results of those test animals that were IgE antibody negative ($\log^2$ total IgE titer<5),[20] were not used for subsequent statistical analysis.

Statistical Analysis

Data were analyzed using the SigmaStat statistical software package (SPSS, Chicago, Ill). For anaphylactic scores, rectal temperature, and histamine levels, the differences between the groups were analyzed by the Kruskal-Wallis 1-way ANOVA on ranks followed by the Mann-Whitney rank-sum test because the data failed to pass the normality test. For IgE levels, the differences between the groups were analyzed by 1-way ANOVA followed by the Bonferroni t test for all pairwise comparisons because the data passed the normality test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Anaphylactic Symptoms Scoring after PNE-challenges

Four weeks post-intervention period followed by ig PNE-challenges, all rats in positive control and sham-treated groups exhibited anaphylactic reactions (median score: 3, 3). In contrast, only 2 out of 7 quercetin–treated rats and 0 out of 7 naive rats developed anaphylactic symptom (median score: 0). Statistical significances in anaphylactic symptom scores between the quercetin–treated and the other sensitized groups were obtained (Table 1).

Table1. Clinical signs of anaphylactic reactions after oral PNE*-challenges in test rats.

Group	Anaphylactic symptoms scores Median (range)	N/total
Positive control*	3 (2-4)	7/7
Sham**	3 (2-4)	7/7
Quercetin	0 (0-1)	2/7
Naive***	0 (0-0)	0/7

Rats in each group were challenged intragastrically with CPE† following 4 w‡ from the initial quercetin intervention. Clinical signs of anaphylaxis were evaluated 30-40 min after the second challenge dose administration; *P < 0.000 QRC§-treated vs. positive control, **P < 0.000 QRC-treated vs. sham-treated rats, ***P < 0.873 QRC-treated vs. naive rats.

*. Peanut extract

†. Crude peanut extract

‡. Week

§. Quercetin

Variations in Rectal Temperatures

20-25 min after the first ig challenge dose, rectal temperatures showed a drop of 2 to 4 ºC nearly in all the sensitized rats before the initiation of interventional period but, 4 weeks post-intervention, the alteration pattern of them was different from expected trend and as a result, needs further investigations in order to declare any uncomplicated promising scientific information (data not shown).

Histamine Release Measurement after PNE-challenges

As shown in Figure 1, 25-30 min after the second ig PNE-challenge dose, plasma histamine levels were markedly lower in quercetin–treated group compared with positive control and sham-treated groups (12.5 ng/mL in quercetin–treated group vs 99.45 ng/mL in positive control and 80.07 ng/mL in sham–treated groups).

Wheal Reactions Analysis after PNE-challenge

At read time, abdominal surfaces of the test rats in positive and sham-treated groups showed up a wheal reaction as a blue area close to/greater than 1 mm in diameter, whereas quercetin completely prevented the same reactions in the treatment group (Figure 2).

Assessment of Vascular Leakage after PNE-challenge

Footpads of the rats in positive control and sham-treated groups exhibited extensive vascular leakage shown by blue color, whereas footpads of the quercetin–treated group were normal (Figure 3).

Trend of Variations in Total Serum IgE Levels

Quercetin treatment was initiated one week after the sensitization period, at which time PN-induced total serum IgE levels had remarkably been elevated, on the average, in all sensitized rats (mean ± SEM; 342.65 ± 18.72 ng/mL in quercetin–treated group, 338.64 ± 13.67 ng/mL in sham group, and 330.44 ± 8.11 ng/mL in positive control rats.

PN-induced IgE levels were not different between sensitized groups before treatment, but were significantly lower in quercetin–treated rats compared with the sham-treated and positive control rats at week 4 end of intervention period (Figure 4).

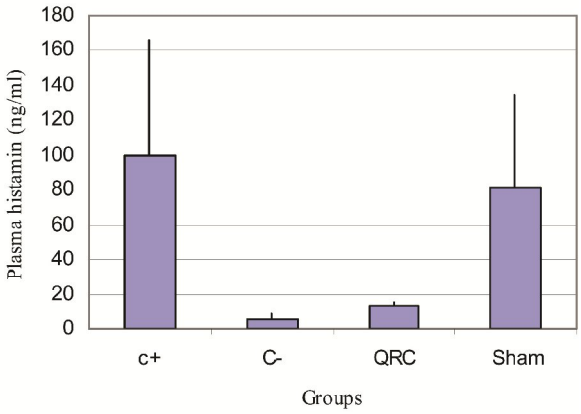
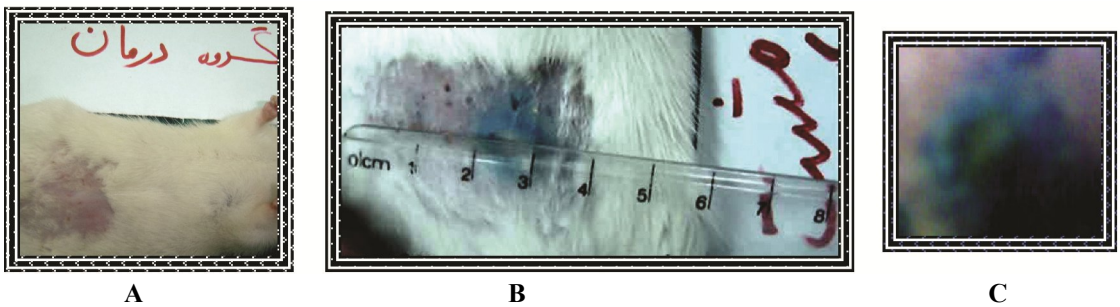
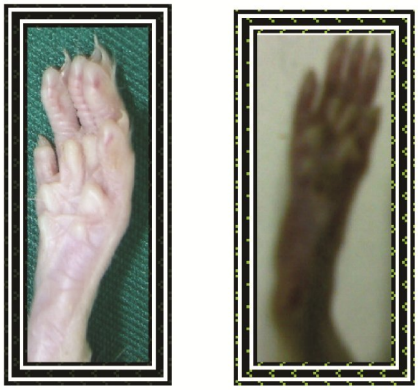


Figure 1. Plasma histamine levels after a quercetin-intervention period of 4 w followed by ig PNE-challenges in all groups of rats. Blood specimens for plasma histamine levels were obtained 25-30 min after the second challenge dose administration and were determined by using ELISA. Quercetin completely abrogated histamine release. Data have been given as means ± SD for each group (7 rats, each). W: week, Ig: intragastric, PNE: Peanut extract, ELISA: Enzyme-linked immunosorbent assay, SD: Standard deviation, C+: positive control; C-: naive; QRC: quercetin. *P < 0.004 QRC-treated vs positive control rats, **P < 0.029 QRC-treated vs sham-treated rats and ***P < 0.989 QRC-treated vs naive rats.



A **B** **C**

Figure 2. Photographs illustrate skin tests after id CPE administration at week 4 end of intervention period; Marked blue-colored bumps in the abdominal skins of the positive control and sham-treated rats (B,C), but not in those of quercetin-treated rats (A). Results represent 4 rats from each group.



A B

Figure 3. Photographs illustrate rats' footpads after ip CPE administration at week 4 end of intervention period; Quercetin prevented vascular leakage (A), marked vascular leakage (blue) was seen in the positive control and sham-treated rats (B). Results represent 3 rats from each group.

DISCUSSION

To validate the efficacy of the flavonoid quercetin and to characterize its potential role of suppression of allergic reactions in an In vivo system, we were prompted to follow the animal model system as a model for PN allergy.

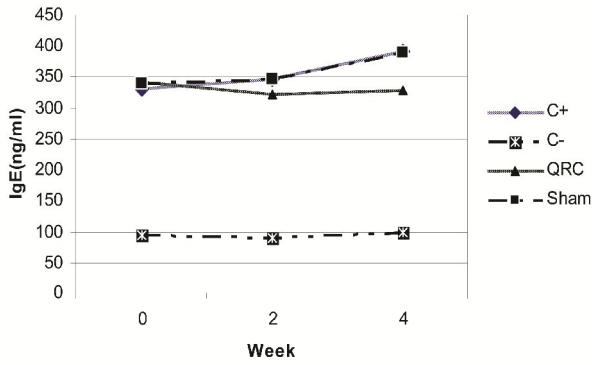


Figure 4. Levels of PN-induced IgE. Sera from all groups of rats were obtained 1 w post-sensitization period (w=0) at which time quercetin treatment was initiated, and biweekly afterwards. PN-induced IgE levels were determined by using ELISA. Data have been given as means ±SD for each group (7 rats, each). PN: Peanut, IgE: Immunoglobulin E, W: week, ELISA: Enzyme-linked immunosorbent assay, SD: Standard deviation, C+: positive control; C-: native; QRC: quercetin.
w=0;*P < 0.000 QRC-treated, positive control, and sham-treated rats vs naive rats.
w=2;*P < 0.000 QRC-treated, positive control, and sham-treated rats vs naive rats.
w=4;*P < 0.014 QRC-treated vs positive control rats, **P < 0.019 QRC-treated vs sham-treated rats and ***P < 0.000 QRC-treated vs naive rats.

Taking to account the homeostatic similarities between rat and man, previous studies have suggested that the Brown Norway Rats (BNRs) are the most pertinent model for human allergic diseases. Here, based on our findings, we dare report, for the first time, that the wistar model may even be more predictive of human PN (extract)-induced allergic responses.

Notably, after sensitization period followed by daily-repeated gavaging, quercetin completely abrogated the PN-induced anaphylactic reactions following ig challenges. The intervention effects were confirmed by a significant decrement in plasma histamine levels compared with positive control (p=0.004) and sham groups (p=0.029) and, in anaphylactic symptom scores compared with positive control and sham groups (p=0.000), as well as, by negative id-and ip-challenges outcomes, also through a downward trend in total serum IgE secretion compared with positive control (p=0.014) and sham groups (p=0.019) subsequent to at least, four weeks of quercetin administration.

The end results of the present study are in agreement with those of several in vitro studies which have suggested that quercetin (as its well-known, mast cell-stabilizer analogue; cromolin sodium) can inhibit mast cell-mediated anaphylactic reactions.[15,17,21-25]

Underlying mechanisms may be several; not only does quercetin possess good radical-scavenging abilities,[26-29] but it may also be capable of inhibiting or reducing the activity of inflammatory enzymes (Cyclooxygenase and Lipooxygenase).[30,31] Reviewing the fact that "Inflammation" is a radical-derived, as well as, a radical-producing process, the extant outcomes are quite rationale and plausible. On the other hand, the preventive effect observed in this research, possibly is related to down-regulation of gene expression argued in plants,[32] rather than to inhibition of the enzymes activity itself. If the former came true, supposedly, quercetin can modulate the expression of FcεRIs on basophils[33] and mast cells and thereby, affect their release from the effector cells, and so forth.

Other mechanism of quercetin action might be the modulation or inactivation of PI3-kinase (a key enzyme involved in effector cells activation).[34]

Repairing/healing the damaged gut epithelial cells and thereby lowering the gut permeability and consequently, preventing the allergen(s)/helper T cells subset2 (Th2) encountering, can be another mechanism of action that gives a good reason also for downward

trend of IgE antibodies secretion and the subsequent reactions.

In sum, to our knowledge, we are the first to show a significant reduction in some well-established anaphylactic reaction parameters by quercetin intervention in an In vivo system in a wistar model of PNE-induced, IgE-mediated food allergy.

Duration of the intervention period was chosen based on literature data. Four weeks of quercetin supplementation proved to be sufficient for inducing antioxidant defense mechanisms, as was indicated by significant decrement in plasma histamine levels. Possibly, even though two weeks of treatment may be sufficient to decrease the plasma concentration of histamine, but it is too short a period to induce any biological effects at the cellular level. It must be borne in mind that, as reported by Takubo, et al.[35] and Inove, et al.,[36] the pharmacological activity of a natural product might be enhanced by repeated administrations. However, it remains to be clarified definitely, whether it also exerts anti-allergic properties in the human body.

At the end, it is of great importance to note that, the implemented tests for Liver and Kidney function analysis, all were within the normal range (data not shown), and circumstantially Quercetin has no apparent toxicity at least, at doses were applied in the present study.

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