Effect of combination of Bromelain and Quercetin extracted from pineapples and fenugreek in mice induced arthritis

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Abstract

This study revealed that bromelain from pineapple and quercetin from fenugreek mixture were active in reducing the effect of arthritis the body diameter measurements within day 4 until the day 10 of peak of induction of arthritis reached the maximum at 5.8 for CRP (mm/hr) ESR (mg/Dl) and reduced it to nearly the normal value 4.3 for CRP and 1.9 for ESR at day 30 with concentration 350 mg/kg of (bro, quer) and within day 4 until the day 10 of peak of induction of arthritis reached the maximum at 2.8 mm of paw volume and reduced it to nearly the normal value 2.9 for 300 mg/kg of (bro, quer) and 2.6 for 350 mg/kg (bro, quer) at day 30 with concentration 350 mg/kg , While within day 0 until the day 10 of peak of induction of arthritis reached the maximum at 83 ng/dl for IgG2 and 70 ng/dl for IgG1 and 40 IgG reduced it to nearly the normal value 68 ng/dl of IgG2 for 350 mg/kg of (bro, quer) and 57ng/dl of IgG1 and 39 ng/dl of IgG for 350 mg/kg (bro, quer) at day 30 with concentration 350 mg/kg .Also within day 0 until the day 10 of peak of induction of arthritis reached the lowest body weight at 18 mg of paw volume and increasing it to nearly the normal weight 19mg for 300 mg/kg of (bro,quer) and 21 mg for 350 mg/kg (bro, quer) at day 30 with concentration.

Keywords: Bromelain(bro), Quercetin (quer), Arthritis.

1. Introduction

Chronic autoimmune disease known as rheumatoid arthritis (RA) is brought on by a mix of hereditary nongenetic and environmental factors. RA damages and malfunctions joints by attacking bone and cartilage Pain, atrophy, joint deformation, bone erosion, and osteoporosis are caused by an over induction of B and T lymphocytes with macrophages, synovial-like fibroblasts, matrix metalloproteinase (MMP) release, and the production of the cytokines interleukin IL1. Interleukin-6 (IL6) (Castro-Santos and Díaz-Pena, 2016). Finding an efficient anti-inflammation medication for arthritis treatments without side effects is an important topic of research., numerous drawbacks as it adversely affects normal parts results in several toxicities. (Vingsbo *et al.*, 1996; Flora, 2007). Fruits and vegetables include a flavonoid called quercetin, which has antioxidant; anti-inflammatory properties. By preventing the production of cytokines, lowering lipopolysaccharide of induced cyclooxygenase, and inhibiting factor of nucleus and quercetin reduces the clinical symptoms of arthritis .It suppresses the mobilization of neutrophils and macrophages; the proliferation of synoviocytes, the current investigation assessed how quercetin affected the synovial inflammation by reducing the activity of the ADA enzyme in a rat model of RA. Additionally, quercetin's combined anti-inflammatory effects. Because of its anti-inflammatory and analgesic qualities. A crude of aqueous extract made from the

pineapple plant's stem or fruit is bromelain, contains a variety of proteolytic enzymes, and has demonstrated potential health benefits. At the moment, sports injuries and acute inflammation are treated with bromelain (Mirshafiey and Mohsenzadegan, 2008; Hitchon and El-Gabalawy, 2004; Hemshekhar *et al.,* 2010).

2. Material and Methods

Extraction and characterization of quercetin from an ethanolic extract of fenugreek Dryed and powdered fenugreek seeds were combined with 50 g of 70% ethanol to make an extraction ,bromelain extraction from pineapple by ultra-filtration, centrifugation finally lyophilization, chemical used Na2S- H2S-sodium cyanide and others proceeding the action as stimulatory agents(Mamo and Assefa, 2019) Filtered extracts from the collected extracts were then concentrated with rotary evaporator then kept at refrigerator. In adding 10 g of Fenugreek extract for silica gel column, the quercetin was extracted (60–120 mesh). Hexane at a concentration of 100% was used to start the elution process. Next, polarity was enhanced using hexane, ethyl acetate, and ethanol. The fractions that eluted were collected, dried, and ground into yellow powder. Additionally, UV Spectra at 257 nm, 307 nm, and 432 nm were recorded. Additionally, use a spectrophotometer (Angeby-Möller *et al.,* 2008; Ängeby Möller *et al.,* 2018)

2.1. Experimental approach

Thirty albino mice divided into a following four groups 1.5 ml of saline was subcutaneously injected into the right hind foot paw of the mice for Grp1 (Normal control); Grp2 (rheumatoid arthritis): To cause arthritis in mice 0.1ml of complete Freund's adjuvant (CFA) was subcutaneously injected into area of the right hind foot paw. Inflammation appeared soon after the injection for seven days later.

Mice were intraperitoneally injected twice a week for three weeks with 100 l of 300 mg/kg of Grp3 (bro, quer 30). Grp4 (bro, quer 35):mice were injected with 100 µl 350 mg/kg of quercetin bromelain three time for per week for 3 weeks.

Changes of body weight and paw thickness. On days 0 through 30, the paw thickness was measured via digital Vernier caliper and represented in mm. Days 1 through 30 were used to estimate body weight changes. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), IGg, IGg1, and IGg2 were tested as part of the biochemical analysis.

2.2. Statistics

Graph Pad was used to determine the relevance of the data, which were given as mean SEM. Using a repeated two-way ANOVA test and a multiple comparison test, data were compared between mice at the same time point; the mice comparison was significant at p 0.05.

3. Results and Discussion

The results as shown in Figure-1 revealed the within day 4 until the day 10 of peak of induction of arthritis reached the maximum at 5.8 for CRP (mm/hr) ESR (mg/Dl) and reduced it to nearly the normal value 4.3 for CRP and 1.9 for ESR at day 30 with concentration 350 mg/kg of (bro, quer).

The results as shown in Figure-2 revealed the within day 4 until the day 10 of peak of induction of arthritis reached the maximum at 2.8 mm of paw volume and reduced it to nearly the normal value 2.9 for 300 mg/kg of (bro, quer) and 2.6 for 350 mg/kg (bro, quer) at day 30 with concentration 350 mg/kg.

The results as shown in Figure-3 revealed that within day 0 until the day 10 of peak of induction of arthritis reached the maximum at 83 ng/dl for IgG2 and 70 ng/dl for IgG1 and 40 IgG reduced it to nearly the normal

value 68 ng/dl of IgG2 for 350 mg/kg of (bro, quer) and 57ng/dl of IgG1 and 39 ng/dl of IgG for 350 mg/kg (bro, quer) at day 30 with concentration 350 mg/kg. The results as shown in Figure-4 revealed that within day 0 until the day 10 of peak of induction of arthritis reached the lowest body weight at 18 mg of paw volume and increasing it to nearly the normal weight 19mg for 300 mg/kg of (bro, quer) and 21 for 350 mg/kg (bro, quer) at day 30 with concentration 350 mg/kg.

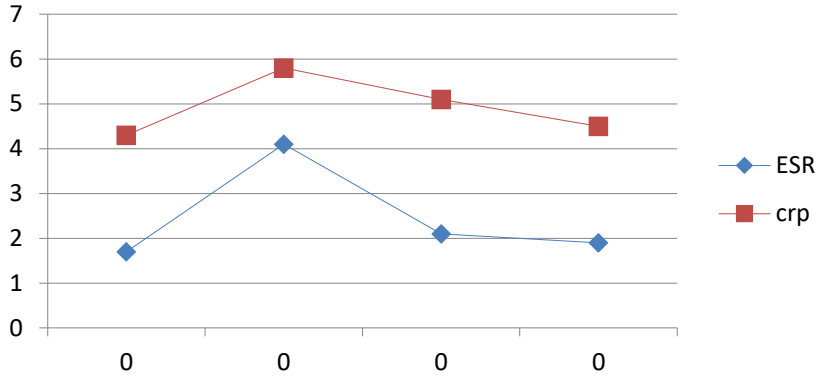

Figure 1: the effect of bromelain and quercetin on arthritis by values of ESR and CRP

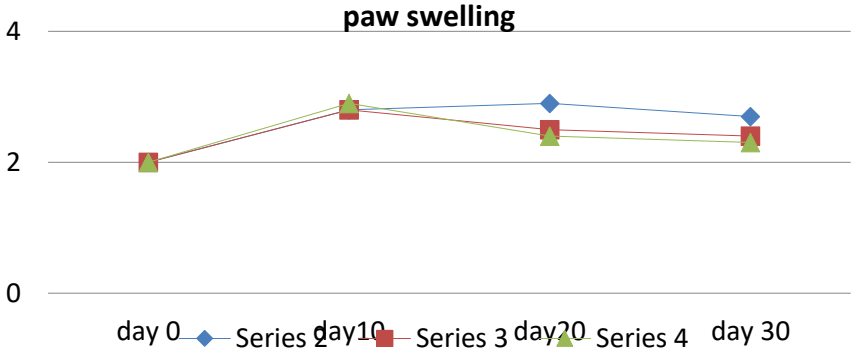

Figure 2: the effect of bromelain and quercetin on arthritis by values of paw swelling

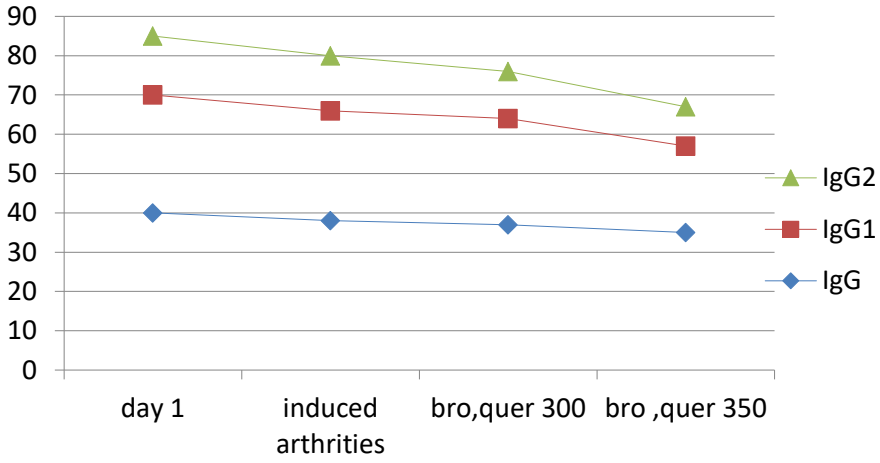

Figure 3: the effect of bromelain and quercetin on arthritis by values of IgG,IgG1,IgG2

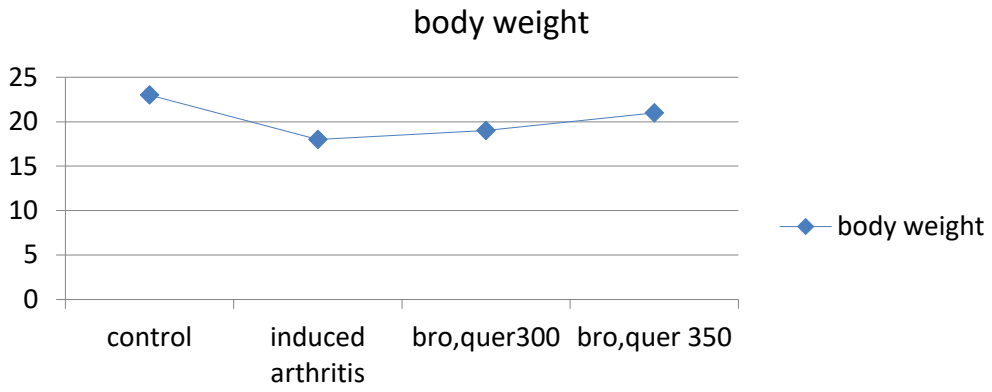

Figure 4: the effect of bromelain and quercetin on arthritis by values of Body weight

Anti-oedematous and antithrombotic and fibrinolytic effects, proved by bromelain having a number of advantageous qualities, including anti-inflammatory and analgesic activity bromelain bromelain's anti-inflammatory effects are mediated by the following mechanisms: increasing the serum fibrinolytic activity, decreasing plasma fibrinogen levels, reduces vascular permeability, consequently, reduces pain), mediating prostaglandin levels and Nevertheless, many of studies are low quality, and more information is required to elucidate the precise mechanisms of its Human inflammatory pain (Svensson *et al.*, 2012; Koyama *et al.*, 2014; Gillooly *et al.*, 2017), human urogenital inflammation, and a number of animal inflammatory models are among the conditions where melanin exhibits analgesic effects. Both effects on pain mediators like bradykinin (and its indirect effects through its anti-inflammatory (such as a decrease in oedema, debris, and immune complexes), which reduce pain, are regarded to be the cause of its analgesic characteristics.

The majority of trials evaluating bromelain for osteoarthritis have either been open studies or equivalency studies intended to compare the efficacy and safety of the medication with that of conventional NSAIDs, used placebos to evaluate the effectiveness of bromelain in treating osteoarthritis. A studies conducted up to this point will be reviewed in the sections that follow. It is challenging to directly compare these trials because different bromelain dosages or formulations were used. Most studies have methodological flaws that make (Sudakov, 1992; Ita *et al*., 2013).

Quercetin's impact on rheumatoid arthritis The pathogenesis of RA is complicated and is still being studied. Clinical treatment focuses mostly on symptom relief and inflammation reduction. The possible health benefits of quercetin have attracted a lot of study attention. As a result, the anti-rheumatoid activity of quercetin is summarized in terms of its analgesic, anti-inflammatory, and impact on experimental rheumatoid animal models. Mechanism of action and anti-inammatory effect Rheumatoid illnesses are heavily reliant on inflammation (Ubani, 2009; Williams and Tabas, 2005).

Conclusions

Bromelain from pineapple and quercetin from fenugreek mixture were active in reducing the effect of arthritis the body diameter measurements on the peak of induction of arthritis reducing reading to nearly the normal value for CRP and ESR at day 30 with concentration 350 mg/kg of (bro, quer) , paw volume reduced value at 350 mg/kg (bro, quer) , while reducing values of IgG2 at mg/kg of (bro, quer) and

IgG1 and IgG for 350 mg/kg (bro, quer) at day 30 with concentration 350 mg/kg bady weight was nearly the normal for 350 mg/kg (bro,quer) at day 30. The bromelain and quercetin are more effectiveness with combining each other in the concentration 350mg/kg for arthritis.

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تأثير خليط البرومالين والكيرستين المعزولين من الاناناس والحلبة على التهاب المفاصل المحفز في الفئران

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الخلاصة

كشفت هذه الدراسة ان خليط البرومالين المعزول من مستخلص الاناناس والكيرستين المعزول من استخلاص الحلبة لها تأثير فعال في خفض تاثيرات التهاب المفاصل ملاحظا خلال قياسات الجسم خلال 4 الى 10 ايام من التحفيز لالتهاب المفاصل حيث وصلت القيم لأقصاها عند CRP (ملم/ساعة)وESR (ملغ /ديسيلتر) ثم انخفضت المقاييس لهما الى 4.3 ,1.9 على التوالي خلال 30 يوما من بداية التحفيز باستخدام 350 ملغ /كغ من الخليط من البرومالين والكيريستين وانخفض حجم المفصل الى 2.4 ,2.6 ملم تابعا باستخدام التراكيز 350 ملغ/ كغ خلال 30 يوما من التحفيز.عند ذروة التهاب المفاصل وصلت القراءات إلى الحد الأقصى عند 83 نانوغرام / ديسيلتر لـ IgG2 و 70 نانوغرام / ديسيلتر ل IgG1 و 40 IgG ولوحظ انخفاضها إلى 68 نانوغرام / ديسيلتر من IgG2 عند استخدام 350 ملغ / كغ من خليط البرومالين والكريستسين و 57 نانو غرام /ديسيليتر لـ IgG1 و 39 نانوغرام / ديسيلتر لـ IgG عند استخدام الخليط بتركيز 350 ملغ / كغ عند اليوم. عند ذروة تحريض التهاب المفاصل سجلت الفئران المختبرية إلى أدنى وزن للجسم عند 18 ملغ و ثم عادت إلى ما يقرب من الوزن الطبيعي 19 ملغ و 21ملغ باستخدام الخليط 300 و350 ملغ / كغ للخليط خلال اليوم الـ 30 من بداية تحفيز الالتهاب