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In Vivo Synergistic Anti-Inflammatory Action of Rutin and Quercetin using Rat Model

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ABSTRACT
Inflammation is a complex biological process concerning various cellular and molecular mediators, often leading to significant damage to the tissue. Anti-inflammatory medications have become crucial for treating inflammatory disorders, but the development of drug resistance and undesirable side effects has made the quest for complementary or alternative therapies important. This study aimed to investigate the possible synergistic anti-inflammatory effects of quercetin as well as rutin, two flavonoids with established antioxidant properties, when combined with Diclofenac in an in vivo model. Rats were used in the study to assess the anti-inflammatory possessions of quercetin and rutin utilizing a variety of models, including paw edema produced by carrageenan, climbing staircases, and motility tests. The findings demonstrated quercetin's and rutin's strong anti-inflammatory properties, with the combination therapy having beneficial benefits. Rutin and quercetin together significantly reduced paw oedema in the carrageenan- influenced paw oedema model, almost equal to the effectiveness of diclofenac. In the staircase climbing activity, significant recovery was seen when quercetin and rutin were combined; this indicated a synergistic effect and was almost as effective as diclofenac. In the motility test, the anti-inflammatory properties of quercetin along with rutin were separately evident, but the combination significantly reduced inflammation. The results suggest that rutin and quercetin, especially when taken together, are effective anti-inflammatory treatment options that can effectively reduce inflammation and its related mobility deficits. Further exploration and potential clinical application of rutin and quercetin as therapeutic agents for inflammatory conditions are supported.

Keywords: Rutin, Quercetin, Diclofenac, Synergistic anti-inflammatory, Carrageenan- induced paw edema.

INTRODUCTION

Inflammation is a complicated biological reaction that occurs in body tissue to adverse stimuli such as infections, cells that are damaged, or irritants [1]. It is a defence mechanism intended to remove necrotic cells and tissues damaged in the first attack, eliminate the underlying source of cell harm, and start the healing process of damaged tissue. Heat, discomfort, redness of the skin, bulging, and loss of functionality are signs of inflammatory conditions. Inflammation has two stages: acute inflammation and chronic inflammation [2]. Acute inflammation is the body's quick and first reaction to damage, identified by the bloodstream's discharge of plasma and leukocytes especially granulocytes to the affected areas. There are several processes involved in this process, such as enhanced vascular permeability, neutrophil emigration, and vasodilation [3]. Chronic inflammation, on the other hand, is a long-term reaction characterised by a gradual change within the type of cells existing at the region of inflammatory responses, as well as tissue damage and repair by the process of inflammation [4]. Effective inflammatory therapy often includes treating the underlying cause, using anti-inflammatory medications, and changing one's lifestyle to lessen inflammatory reactions [5].
Current inflammatory therapy encompass many pharmacological and non-pharmacological approaches aiming at reducing symptoms while also dealing with underlying causes. Nonsteroidal anti-inflammatory medicines

such as naproxen and ibuprofen reduce inflammation as well as pain, but they also come with hazards including cardiovascular and gastrointestinal disorders. [6]. Corticosteroids, like prednisone, are used to treat severe inflammatory conditions including rheumatoid arthritis and asthma. However, they have substantial adverse effects that include increased risk of infection and weight gain. Corticosteroids also weaken the immune system. While Janus Kinase (JAK) inhibitors offer a different, more specialised treatment for autoimmune diseases, they also carry the risk of serious infections. Anti-rheumatic drugs which are disease- modifying (DMARDs) and biologic medications target certain immune pathways to treat autoimmune illnesses [7].

Numerous plants, including buckwheat, citrus fruits, and berries, contain rutin, often referred to as rutoside or quercetin-3-rutinoside. [8]. Its pharmacological properties are significant and include anti-inflammatory, antioxidant, and vasoprotective effects. Rutin exerts its effects via NF- κB pathway modulation, antioxidant activity, mast cell stabilisation, and inhibition of inflammatory mediator. When rutin is taken orally, it gets absorbed at small intestine, metabolised in liver, and mostly eliminated as urine. [9].

Quercetin, a widely diffused flavonoid found in fruits, vegetables, and grains, has a wide variety of pharmacological actions and has tremendous medical promise [10]. Its many benefits include antioxidant, anti-inflammatory, anticancer, and cardioprotective qualities. Quercetin's pharmacological activities are mediated through a variety of mechanisms. This anti-inflammatory medicine inhibits the production of cytokines which are pro-inflammatory (e.g., TNF-α, IL-6), enzymes (e.g., COX-2, iNOS), and transcription factors (e.g., NF-κB), hence lowering inflammation. [11]. Furthermore, oxidative stress reduction, enhanced endogenous antioxidant defences, and free radical scavenging are some of quercetin's antioxidant properties. These systems protect cells and tissues from oxidative damage caused by a number of medical problems. [12]. Quercetin possesses anticancer effects that reduce tumour cell growth, induce apoptosis, and decrease metastasis through signalling pathways involved in cell survival and death [13].

Carrageenan, a seaweed-derived polysaccharide, is known to induce inflammation in experimental models. It's commonly used to study acute inflammation due to its ability to mimic human inflammatory responses [14,15]. This research focuses on evaluating of Rutin and Quercetin's anti-inflammatory properties in rats with carrageenan-induced paw edema. By comparing their effectiveness to established anti-inflammatory drugs, the study aims to assess their potential therapeutic benefits in treating inflammatory disorders. The research will examine parameters such as inflammation severity and tissue damage to determine the efficacy of the combined treatment regimen with Rutin and Quercetin.

2. MATERIALS AND METHODOLOGY

2.1 Chemicals and Drugs used

Rutin as well as Quercetin drugs were acquired from Unique Biologicals and Chemicals in Kolhapur, India. The standard anti-inflammatory medicine Diclofenac sodium, Carrageenan, and DMSO were obtained from the Pharmaceutics Department of Ashokrao Mane College of Pharmacy in Peth-Vadgaon, Kolhapur.

2.2 Preparation of Solutions

Distilled water was used to make a 1% carrageenan solution. In addition, Rutin and Quercetin were administered orally using DMSO as a solvent [16].

2.3 Equipment

The Orchid Scientific Digital Plethysmometer (Make- Inco, Model- D.S. No-AMCP?2021- 2022/EQ-67) was used to measure the thickness of the rat's inflamed paw. The measurements on the screen of the Digital Plethysmometer were recorded, and the graph was downloaded from the Plethysmometer machine.

2.4 Pharmacological Study

2.4.1 Grouping of Animals

200–250 grams Wistar rats of both sexes were acquired from the National Institute of Bioscience in Pune, Maharashtra, India. At Ashokrao Mane College of Pharmacy in Peth Vadgaon, the animals have been kept in the laboratory of the Department of Pharmacology. In addition to having unlimited access to water and food, and typical environmental settings were maintained for animals. All procedures were carried out in accordance with CPCSEA requirements. In Ashokrao Mane College of Pharmacy, Peth-Vadgaon, Maharashtra, India, the Institutional Animal Ethical Committee approved the study IAEC/01/AMCP/2022-23.

Table 1: Grouping of Animals

Group	No. of Animals	Dose
A	6	Control vehicle
B	6	Carrageenan (0.1 ml of 1% solution)
C	6	Sodium diclofenac (150 mg/kg body weight)
D	6	Rutin 50 mg

E	6	Quercetin 20 mg
F	6	Rutin 50 mg + Quercetin 20 mg

2.5 Paw edema in rats caused by Carrageenan

Six sets of six rats each were used to test the anti-inflammatory activity against acute inflammation: A control vehicle was provided to Group A, which acted as the control. Group B, Group C, Group D, Group E, and Group F were administered with various drugs. Carrageenan was administered to Group B in order to produce inflammation. Group C received Diclofenac sodium, an anti-inflammatory medication. Group D was injected with rutin, a natural antioxidant. Group E was injected with quercetin, a natural antioxidant. Finally, Group F received both rutin and quercetin. Each group had six animals.

2.6 Anti-inflammatory effect

The carrageenan-induced rat paw edema experiment was used to quantify the anti-inflammatory effect. The anti-inflammatory effectiveness was evaluated using the carrageenan-induced rat paw oedema research. 100 µl of a newly made 1% solution containing the edematogenic agent carrageenan along with distilled water was sub-plantarly injected into each rat's right hind paw to cause edema in all but group A of rats. Group A has been preserved as a distinct vehicle control. Thirty minutes before carrageenan injection, animals in group B, group C, group D, group E as well as group F received single doses of medication and vehicle cultures. A Digital Plethysmometer was used to measure the paws' thickness in order to determine the extent of inflammation at time 0, immediately after the carrageenan injection, and at 1, 2, 3, 4, and 24 hours
[17].

2.7 Activity test for stair climbing

For a week, animals who were fasting overnight were trained to ascend a staircase with steps that were 3 cm, 6 cm, 9 cm, 12 cm, as well as 15 cm in length. The second step was kept with water, while the third was filled with food. Rats in the aforementioned groups were given a climbing ability score of 0 if they did not climb, 1 if they climbed onto step 1, 2 if they climbed onto step 2, 3 if they could climb three steps, 4 if they could climb four stairs, and 5 if they could climb all five steps [18].

2.8 Test for Motility

The motility pattern of rat was monitored for five minutes; if the rat did not move, it received a score of zero; if it walked but avoided contacting the inflamed paw's toes to the ground, it received a score of one. 2, if the rat could walk mostly on its own, but with one toe touching the ground; and 3, if it could walk without much trouble [19].

2.9 Statistical Analysis

The statistical studies were all bring out utilizing GraphPad Prism 5.0. The animal experiment findings were presented as Mean ± SEM. To govern the overall impacts of the therapies, one-way analysis of variance (ANOVA) was utilized in the statistical investigation of the data. Tukey's Multiple Comparison Test was then performed, and values of $P > 0.05$ were deemed statistically non-significant, even though values of $P < 0.05$ were deemed statistically significant. And the Kruskal-Wallis test was utilized to compare the groups, and the median scores for the motility and stair ascending ability tests were reported.

3. RESULT AND DISCUSSION

3.0 Result

The anti-inflammatory activities of quercetin and rutin were studied within rats using a model of paw oedema caused by carrageenan. The groups treated with quercetin and rutin had paw oedema volumes that were considerably lower than those of the control group, according to the data.

3.1 Carrageenan induced inflammation

A progressive oedema was brought by inserting carrageenan into the hind paw, and it peaked in just one hour. For Group A, the animal's paw bulkiness was 0.2000±0.02582 ml at t = 0 and remained steady afterwards 24 hours. On each hour, the animals in Group B showed thicker paws, which was significant at $P < 0.05$. The thickness was 0.4500±0.02236 ml at 0 h and 1.550±0.1176 ml at t = 1 h. After twenty-four hours, the thickness measured 3.733±0.04944 ml. Paw thickness increased by 0.4833±0.03073 ml (t = 0 h), 1.333±0.06146 ml (t = 1 h), 0.7833±0.03073 ml (t = 2
h), and 0.6000±0.03651 ml (t = 4 h) in animals in group C. After the fourth hour, it dropped to 0.3833±0.03073 ml at t = 24 hours. On 0 hours, the paw thickness of Group D animals was 0.5500±0.02236 ml; by the end of the first hour, it had increased to 1.600±0.05774 ml. It dropped after the second hour to 1.267±0.03333 ml, 1.067±0.03333 ml, and 0.8000±0.03651 ml at the completion of 4 hours and 24 hours, respectively. Group E animals paw thickness was 0.6000±0.02582 ml at 0 hours, but at the end of the first hour, it had increased to

1.650±0.04282 ml. Following the second hour, it dropped to 1.400±0.05774 ml, 1.150±0.04282 ml, and 0.7833±0.03073 ml at the conclusion of 4 hours and 24 hours, respectively. By 0 hours, Group F paw thickness was 0.5000±0.03651, but at the end of the first hour, it had increased to 1.517±0.03073 ml. Following the second hour, it dropped to 1.067±0.04216 ml, 0.7833±0.03073 ml, and 3.79 ± 0.206 ml at the completion of 4 hours and 24 hours, respectively. (Table 2)

Thus, paw thickness decreased statistically significantly ($P < 0.05$) in Groups D, E, and F. It was determined that these values were significant statistically at $P < 0.05$. (Fig2).

Throughout the experiment, a notable decrease in hind paw inflammation was observed upon oral administration of quercetin and rutin. After 24 hours of therapy in this regard, rutin and quercetin in combination were superior, followed by just one.

Table 2: Results of Anti-inflammatory activity of Paw thickness in Rats.

Group No.	Treatment Groups (mg/kg)	Paw Thickness of Wistar Rats (in ml)					
		Before Inj.	After Inj. (0 Hour)	1 Hr	2 Hr	4Hr	24 Hr
A	Control	0.2000±0.02582	0.2000±0.03651	0.2333±0.03333	0.2167±0.03073	0.2500±0.02236	0.2667±0.02108
B	Carrageenan induced	0.2500±0.02236	0.4500±0.02236	1.550±0.1176	2.433±0.08433	3.133±0.09545	3.733±0.04944
C	Diclofenac Sodium	0.2167±0.03073	0.4833±0.03073	1.333±0.06146	0.7833±0.03073	0.6000±0.03651	0.3833±0.03073
D	Rutin	0.2667±0.02108	0.5500±0.02236	1.600±0.05774	1.267±0.03333	1.067±0.03333	0.8000±0.03651
E	Quercetin	0.2333±0.02108	0.6000±0.02582	1.650±0.04282	1.400±0.05774	1.150±0.04282	0.7833±0.03073
F	Rutin + Quercetin	0.2167±0.03073	0.5000±0.03651	1.517±0.03073	1.067±0.04216	0.7833±0.03073	0.5333±0.03333

3.2 Stair climbing activity

Carrageenan caused Group B rats to become hyperalgesic. The animal in Group A had the highest score, 5 ± 0.00 (significant statistically; $P < 0.05$), whereas its staircase climbing activity was 1.167± 0.3073. The animals in Group C displayed a score of 3.833 ± 0.1667. The stair climbing scores of Group D, E and Group F were considerably raised by Rutin,Quercetin and both in combination treatments, respectively, by 2.333± 0.2108 , 2.333± 0.2108 and 3.667 ± 0.2108 significant at $P < 0.05$).. The animals in Group B scored lower than those in Groups A, C, D, E. and F Fig.1

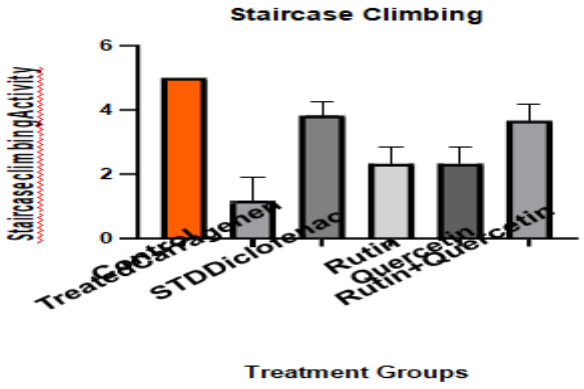

Fig-1 various medications' effects on the lesions of staircase climbing activity scores linked with inflammation caused by carrageenan. The subplantar superficial layer of the right hind paws was injected with 100 µl of a freshly made, 1% solution of the edematogenic agent carrageenan in distilled water to cause edema. Thirty minutes before to injecting the edematogenic substance carrageenan, the medications were given orally. At twenty-four hours, stair ascending activity was noticed. For each group, the particular are shown as Mean ± SEM of 6 rats. Group A: Control; Group B: Carrageenan control; Group C: Diclofenac sodium (150 mg/kg body weight) Group D Rutin 50 mg, Group E: Quercetin 20 mg and Group F Rutin 50 mg + Quercetin 20 mg was administered as the reference standard anti-inflammatory medication to the positive control significant is *P <

0.05, and highly significant is ***P < 0.001.

3.2 Motility Test

The motility score was used to assess the rats' walking ability to ascend the staircase at the period of maximum inflammation. The motility scores of the animals in Group D, E and Group F were 2.333 ± 0.2108, 2.333 ± 0.2108 and 2.667±0.2108, respectively (significant at P < 0.05). The animals in Group A had the highest motility score, which was 3 ± 0.00, while the animals in Group B had the lowest value, which was 0.3333 ± 0.2108. The motility score of the animals in Group C was 2.833 ± 0.1667. Animals in Group B had a lower motility score than those in Groups A, C, D, E and F. Fig.2

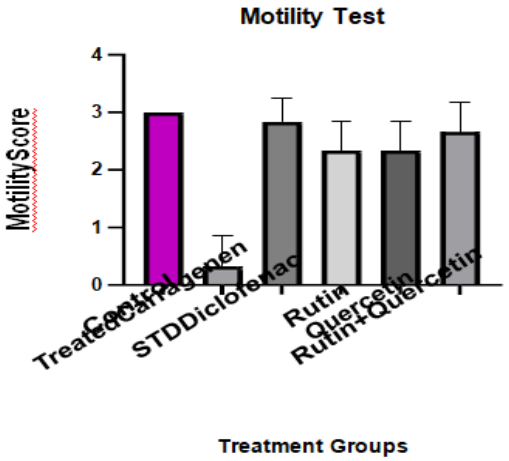

Fig-2 By administering 100 µl of a recently made, 1% solution of the edematogenic substance carrageenan in distilled water under the right hind paws' subplantar surface area, the effect of different treatments on the injury in mobility associated with edema was investigated. Thirty minutes before to injecting the edematogenic agent carrageenan, the drugs were taken orally. At 24 hours, the motility score was noted. Results are expressed as the Mean ± SEM of 6 rats in every group. Groups A, B, C are the control, carrageenan control, and Diclofenac sodium respectively. Groups D,E and F were given Rutin 50 mg., Quercetin 20 mg and Rutin 50 mg + Quercetin 20 mg Group C was given diclofenac sodium 150 mg/kg body weight, which was used as the standard anti-inflammatory pharmacological reference. P < 0.001*** Very significant, P < 0.01** Very significant, and P < 0.05 * Significant

4. DISCUSSION

The typical model used in experiments to simulate acute inflammation is hind paw edema caused by carrageenan. Since carrageenan has no apparent systemic effects and is not known to be antigenic, it is the preferred logistics agent for testing anti-inflammatory medications. Furthermore, there is a high degree of reproducibility in the experimental model. A potent substance called carrageenan is employed to release pro- and inflammatory mediators. [20] Edema brought on by carrageenan is a biphasic reaction [21]. Following the phlogistic agent injection in the initial hours, histamine, sero-tonin, and kinins are released, mediating the first phase [22]. In contrast, the release of chemicals similar to prostaglandins within 2-3 hours is the focus of the IInd stage. Both the clinically beneficial steroidal and nonsteroidal anti-inflammatory agents can be used to treat the IInd stage of inflammation [23]. The primary cause of acute inflammation is prostaglandins. Anti-inflammatory cytokines likewise as IL-10, IL-4 and IL-13 inhibit prostaglandin formation and maintain the levels of Coxygenase-2. The enhanced synthesis of prostaglandins is caused by coxygenase-2 [25.26]. The anti-inflammatory substance that Rutin, Quercetin. may have is what blocks prostaglandins and inflammatory pathways. Rutin, Quercetin and Rutin Quercetin combination shown a remarkable depletion in the thickness of the rat paws (Group D, Group E and Group F) in this model of inflammation due to their extremely consistent anti-inflammatory effect. Past studies of many researcher have also suggested Rutin and Quercetin has anti-inflammatory properties. Although both the lipoxygenase and cyclooxygenase pathways are essential to the inflammatory process, lipoxygenase inhibitors are less successful in preventing inflammation caused by carrageenan than cyclooxygenase inhibitors. It's possible that the cyclooxygenase that produces prostaglandins was suppressed by Rutin Quercetin [27]. It's possible that oral administration of Rutin and Quercetin markedly suppressing the activation of tumor necrosis factor-α (TNF-α), cyclooxygenase (COX-2) and nuclear factor-kappa B (NF-κB) IL-1β, IL-6 and PGE2 [28]. Most of researchers suggested that future research should concentrate on the phytochemicals' synergistic effects as well as the identification of new phytochemicals to aid

in the generation of novel pain treatments and confirmed. Synergistic medication can lower the dosage of medicines and prevent potential harmful side effects during the course of treatment. [29, 30, and 31].
In our study when given Rutin and Quercetin simultaneously showed and we possess documented the synergistic outcome of Q-R upon a number of inflammatory and oxidative indicators, including IL-13, and IL-6, as well as COX-2, nuclear factor-kappa B (NF-κB, and tumor necrosis factor-α (TNF-α). Consequently, the study indicates that Rutin and Quercetin simultaneously is a strong antioxidant and anti-inflammatory drug that has a notable capacity to inhibit mediators of inflammation and oxidative stress. In fact, the medication decreased inflammation more in both Rutin and Quercetin. In our investigation, the Rutin and quercetin in combination exhibits a notably higher reduction in inflammation than the alone. According to the current study's findings, both in combination, along with the medication diclofenac, reduce the initial stage of pawedema caused by carrageenan, indicating that they have valuable effects analogous to those of non- steroidal anti-inflammatory drugs (NSAIDs).

5. CONCLUSION

These studies suggest that in vivo administration of both Rutin and Quercetin, may have effects resembling to those seen with non-steroidal anti-inflammatory medication therapy, likewise diclofenac. Such as with most non-steroidal anti-inflammatory drugs, it is moreover proposed that the mechanism of action of Rutin as well as Quercetin may be linked to the suppression of cyclooxygenase, which facilitates the production of prostaglandins. And when given in combination shows significant synergistic effect.

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