RB SCIENCE
ENTERPRISING BIOSCIENCE RESEARCH
www.rbscience.co.in

E-ISSN: 2456-8244

JOURNAL OF PHARMACOLOGY AND BIOMEDICINE

Evaluation of anti-inflammatory potential of Rutin using *in vitro* models

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Abstract

Rutin is a secondary plant metabolite known to possess excellent antioxidant potential. The present work was undertaken with an objective to evaluate the anti-inflammatory potential of rutin using in vitro models. Inhibition of albumin denaturation and antiprotease action were used as the models for studying the anti-inflammatory action. The capacity of different concentrations of rutin to inhibit protein denaturation of albumin was ranging from 27.33 ± 0.001% to 67.82 ± 0.002 comparable to ibuprofen (standard). On the other hand the capacity of different concentrations of rutin to inhibit serine protease was found to be ranging from 20.36 ± 0.214% to 77.53 ± 0.138 in the assay and therefore reinforces for its tremendous anti inflammatory property.

Keywords: Rutin, albumin denaturation, antiprotease, anti-inflammatory, *in vitro*, chemical mediators

Introduction

Inflammation is a response to stimulation by invading pathogens or endogenous signals such as damaged cells that results in tissue repair or sometimes pathology. It is a fundamental immune response by the host that enables the elimination of harmful stimuli as well as the healing of damaged tissue.[1] Acute inflammation is considered as a part of innate immunity, the first line of host defense against foreign invaders and danger molecules. It may progress slowly into chronic inflammation which may also be caused due a number of diseases like cancer, neurodegenerative disorder and cardiovascular diseases.[2] Owing to the precipitating factors of chronic inflammation, it needs to be treated symptomatically. The majority of drugs used to treat inflammation may be either steroidal or non steroidal (NSAIDs). The NSAIDs act by inhibiting the enzyme COX-1&2 wherein inhibition of COX-2 caused anti-inflammatory action while that of COX-1 leads to the precipitation of undesired effects of NSAIDs like gastrointestinal and renal toxicities.[3]

Owing to these critical adverse effects associated with the NSAIDs, the locus has shifted towards the use of herbal products of anti inflammatory potential. A complete scientific investigation of herbal medicines is required in order to ensure the safety and efficacy of the same. Different models of evaluation of the efficacy of drugs and medicines needs to be studied in order to confirm the potential of these drugs. Several scientific studies on herbal medicines have been carried out for anti-inflammatory action[4-7] and it was found that flavonoids present in plants are involved in disrupting the oxidative pathways thereby causing the anti-inflammatory action of the plant extracts.[8] Rutin is a secondary metabolite of class flavonols present in several plants and has shown promising antioxidant action. It has also been evaluated for its anti-inflammatory potential in animal models.[9,10]

Majority of anti inflammatory agents are evaluated directly by using the *in vivo* methods in animal thereby creating concern among the animal right activists' worldwide. A number of *in vitro* tests are also available for evaluating the effect that the drug molecules may have on inflammation. These models are based on the inhibition of the chemical mediators of inflammation and are considered to be more tedious than the *in vivo* methods and hence are often bypassed by the researchers. The present work was aimed to ascertain the anti-inflammatory potential of Rutin using some *in vitro* models for the same.

Material and Methods

Rutin was purchased from TCI Chemicals (India) Pvt. Ltd and its identity was confirmed by IR spectroscopy prior to use. The purity of the drug was confirmed by the supplier as 94.5%. It was used as obtained without any processing for purification. All other chemicals used in the study were of AR grade and were procured from the local chemical supplier.

Of the several available methods for in vitro evaluation of anti-inflammatory activity two methods viz. inhibition of albumin denaturation and inhibition of protease activity were selected for our study.

Inhibition of albumin denaturation[11]

Preparation of Phosphate Buffer Saline (PBS)

A solution of PBS was prepared by dissolving an accurately weighed quantity of 8 g NaCl, 0.2 g KCl, 1.44 g disodium hydrogen phosphate and 0.24 g potassium dihydrogen phosphate in deionized water to produce 1 L of solution.

The technique of inhibition of albumin denaturation reported by Kumari *et al*[12] was used with slight modifications. The volume of each component of the reaction mixture was reduced to half its volume. Rutin was dissolved in DMSO and appropriately diluted to prepare solutions of 62.5, 125, 250, 500 and 1000 mM concentration. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 μg/mL was used as the positive control. The reaction vessel was filled with 200 μL of BSA, 1400 μL of PBS and 1000 μL of the test solution (rutin). Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of rutin solution.

The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm. The inhibition of percent denaturation of albumin was determined using the following formula:

$$\% \text{ Denaturation inhibition} = (1-D/C) \times 100\%$$

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

Antiprotease action

Preparation of Tris-HCl buffer

An accurately weighed quantity of 121.44 g of Tris was dissolved in 800 mL of distilled water. The pH of the solution was adjusted to 7.0 by addition of appropriate volume of concentrated HCl and the final volume of the solution was made up to 1 L with distilled water.

The technique of antiprotease action reported by Oyedepo *et al*[13] and Sakat *et al*[14] was used with slight modifications. The reaction mixture was prepared with 0.06 mg trypsin, 1 mL 20 mM Tris-HCl buffer (pH 7.0) and 1 mL test sample of different concentrations (100 - 500 μg/mL). The mixture was incubated at 37°C for 5 min followed by the addition of 1 mL of 0.8% w/v solution of casein in water. The mixture was incubated additionally for 20 min. In order to stop the reaction, 2 mL of 70% perchloric acid was added to the mixture. The turbid suspension obtained after the reaction was centrifuged and the absorbance of the supernatant was recorded at 210 nm against buffer as blank. The percentage inhibition

of protease activity was calculated by the following formula:

$$\%\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ contorl} * 100$$

Statistical Analysis

All the experiments were performed in triplicate and the results are expressed as mean ± standard deviation. The difference between the experimental groups was compared by one way ANOVA followed by Dunnets multiple comparison test using Graph Pad Instat software.

Results and Discussion

The anti inflammatory activity was assessed using the in vitro models and the results of each model were statistically evaluated for significance.

Albumin Denaturation Inhibition

All the concentration levels of rutin exhibited inhibition of albumin denaturation (Table 1). The concentration of 1000 mM rutin had shown the greatest inhibition capacity with 67.87 ± 0.002% whereas the lowest inhibition capacity of 27.33 ± 0.001 % was exhibited by 125 mM concentration. The inhibition of 62.5 mM concentration was not significant while the inhibition capacity of the positive control (Ibuprofen) was found to be 68.43 ± 0.001%.

Table 1 Albumin denaturation inhibition activity

S. No	Test Group	Conc	Absorbance	% Inhibition
1	Control	-	0.4153	0
2	Rutin	62.5 mM	0.3216	22.56 ± 0.05
3	Rutin	125 mM	0.3018	27.33±0.001**
4	Rutin	250 mM	0.2647	36.26±0.001**
5	Rutin	500 mM	0.1582	40.23±0.002**
6	Rutin	1000 mM	0.1334	67.82±0.002**
7	Standard (Ibuprofen)	1 µg/ml	0.1311	68.43±0.001**

** $p<0.01$ considered as extremely significant

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis.[15] According to Opie[16], tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity.

The capacity of different concentrations of rutin to inhibit protein denaturation of albumin was ranging from 27.33 ± 0.001% to 67.82 ± 0.002 in the assay and hence provides yet another evidence for its excellent anti inflammatory property. The statistical analysis has shown that there is no significant difference ($p<0.01$) between the mean of the different concentrations of rutin except 62.5 mM

concentration which was not found to be significantly correlating.

Antiprotease action

Rutin was found to inhibit protease activity in all the tested concentrations (Table 2). The highest inhibition capacity was exhibited by rutin solution of 500 µg/mL concentration, inhibiting 77.53 ± 0.138% while the 100 µg/mL rutin solution was able to inhibit only 20.36 ± 0.214% protease activity. Aspirin solution of 100 µg/mL concentration was able to inhibit 48.78 ± 0.211% of protease activity.

Table 2 Antiprotease activity of rutin solution

S.No	Test Group	Conc (µg/mL)	% Inhibition
1	Control	-	0
2	Rutin	100	20.36 ± 0.214*
3	Rutin	200	22.78±0.348*
4	Rutin	300	32.36±0.280**
5	Rutin	400	55.23±0.211**
6	Rutin	500	77.53 ± 0.138**
7	Standard (Aspirin)	100	48.78 ± 0.211**

*p<0.05, **p<0.01

Neutrophils are known to be a rich source of serine protease and are localized at lysosomes. It has been previously reported that leukocytes protease play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by protease inhibitors.[17] Although mast cell protease-dependent proteolysis is critical to host defense against invading pathogens, regulation of these hydrolytic enzymes is essential to limiting self-induced damage as well. Indeed, dysregulated release of mast cell proteases is now recognized to contribute to the pathogenesis of a number of inflammatory conditions including asthma, abdominal aortic aneurysm formation, vessel damage in atherosclerosis and hypertension, arthritis, and ischemia/reperfusion injury.[18]

The capacity of different concentrations of rutin to inhibit serine protease was found to be ranging from 20.36 ± 0.214% to 77.53 ± 0.138 in the assay and therefore reinforces for its tremendous anti inflammatory property. The statistical analysis has shown that there is no significant difference between the mean of the different concentrations of rutin. The inhibition of protease was very much comparable to the standard drug Aspirin used in the study.

Conclusion

The object of the present investigation was to ascertain the anti-inflammatory potential of the plant secondary metabolite, Rutin using in vitro models. Rutin compound has demonstrated promising anti-inflammatory potential by *in vitro* method suggesting the possible mechanism of interfering with the chemical mediators of inflammation. Increasing awareness, promotion and utilization of this flavonol compound will serve as a natural anti-inflammatory compound. A few more *in vitro* models could be studied for establishing the complete mechanism of action of rutin at the cellular and molecular level in treating inflammation.

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