

# Anti-Inflammatory Activity of Quercetin in Acute and Chronic Phases of Inflammation in Guinea Pigs

Warda Mohamed Kaidama\* and Rajesh N. Gacche

Department of Biotechnology, School of Life Sciences, S.R.T.M. University, Vishnupuri, Nanded, Maharashtra, India-431606

## Address for Correspondence

Department of  
Biotechnology, School  
of Life Sciences,  
S.R.T.M. University,  
Vishnupuri, Nanded,  
Maharashtra, India-  
431606.

### E-mail:

[warda\\_m\\_2012@yahoo.com](mailto:warda_m_2012@yahoo.com)

## ABSTRACT

**Objective:** To evaluate the anti-inflammatory activity of flavonoid (Quercetin 20 mg/kg) on acute inflammation (carrageenan-induced paw edema) and chronic inflammation (cotton pellet granuloma) in guinea pigs.

**Methodology:** Studying sample of guinea pigs were divided in two groups. Group I was used as control received 1ml of 5% carboxymethyl cellulose suspension, group II was treated with indomethacin (10 mg/kg) and Quercetin at a dose of 20 mg/kg was given orally to group III. 1.0 ml of carrageenan was injected s.c. to plantar region of right hind paw of each pig. The change in paw volume was measured at 0, 1, 2, 3 and 4 hours intervals. For chronic model of inflammation, sterilized cotton pellets weighing  $50 \pm 1$ mg each, were implanted into both sides of the groin region of each pig, under light anesthesia. Drug treatment was given for 7 days. On the eight day, cotton pellets along with granuloma were removed surgically and wet pellets were weighed, after that dried at  $60^{\circ}\text{C}$  overnight and then the dry pellets weight was taken.

**Results:** The results indicates that, Quercetin at a dose of 20 mg/kg body weight exhibited significant inhibition ( $P < 0.05$ ). The Percent Inhibition with the control, standard (Indomethacin) and the test compound (Quercetin) in the carrageenan induced paw edema model were 0%, 74% and 68% and 0%, 37%, and 34% respectively in cotton pellet induced granuloma method.

**Conclusion:** This study concludes that, Quercetin have significant anti-inflammatory effects in both acute and chronic inflammatory conditions.

**Keywords:** Anti-inflammatory activity, Carrageenan, Cotton pellet granuloma, Quercetin.

## INTRODUCTION

When pathogens or irritation occurs and damage cells, inflammation appears as a defensive agent by the tissue as a response to such above mentioned harmful factors<sup>1</sup>. Inflammation plays two major roles in specifying damage and enhancing treatment of tissues. Although inflammation is beneficial in providing defense against infection invaders, it may become unchecked in case of pathogenesis of chronic inflammatory disease<sup>2</sup>. The basic mechanism of inflammation is that the cells linked with inflammation on membrane to produce acid and several eicosanoids as well as the releasing of lysosomal enzyme. There are two sorts of inflammatory activities acute and chronic which are recognized to be unresolved activities formed by many various compounds of chemical factors such as prostaglandins, leukotriene's and palate motivating agent and so on like this. Anti-inflammatory factors intensify their impact through chain of various methods of action<sup>3</sup>.

The response of acute inflammation is categorized by the rising of vascular permeability and cellular infiltration causing edema composition due to the extra vacation of fluids and proteins accumulation of leukocytes at the inflammatory position for short time<sup>4</sup>.

Chronic inflammation appears if the acute response is not enough to eradicate the agents of pro inflammatory and it compromises increasing of fibroblasts and infiltration of neutrophils with exudation of fluid. It rises by methods of growing increasing cells which can either spread or compose granuloma. Not only this but, it probably also happens because of the insistence of infection or antigen, recurring tissue injury, or a failure of endogenous anti-inflammatory mechanisms<sup>5</sup>.

There are a lot of elements to an inflammatory reaction that can lead to the associated adverse and tissue damaging. The

elements of inflammation play significant role in edema and granuloma composition as well as leukocyte infiltration<sup>6</sup>. Edema existence in the paw is because of a synergism between various inflammatory intermediates that raises vascular permeability and/or the mediators that increase flowing of blood. The level of acute inflammation is identified by carrageenan induced paw edema as it is widely used for such detection<sup>7</sup>. Flavonoids have significant function in maintaining human health and protecting it from any diseases. Furthermore it plays significant role in preventing and fighting allergy, viruses in addition to preventing of the properties of inflammation. This last one constitute an aspect employed for a long time in the Chinese traditional medicine and the cosmetic industry represented by the compound of plant extracts, but recently it has begun to be explored in depth, in order to identify the mechanisms responsible and the possibility for use of flavonoids as anti-inflammatory agents<sup>8</sup>.

Flavonoids are referred to a component of normal substances take part in their chemical compounds various number of hydroxyl phenolic groups. They are existed in sources of vegetal origin (fruits, seeds, roots, flowers, tea or wine). Many different types of flavonoids which are identified and estimated to be no less than four thousands. Most of those varieties and almost all are phenyl benzopyrones of low molecular weight with a main structure composed by two benzene chains linked through a heterocyclic pyrane or pyrone. They form differences in their chemical structure that can simplify the interaction with specific receptor molecules and/or their respective pathways within the cells, such as apoptosis, cell activation to stress and cascades of protein signaling kinases. Based on molecular structure they are divided in

four fundamental groups: flavones, flavanones, flavanols and anthocyanin's<sup>9</sup>.

Quercetin (3,3',4',5,7-Pentahydroxy flavone) is a naturally occurring polyphenol produced by a variety of plant species<sup>10</sup>. It is part of a group of phytochemicals called polyphenolic flavonoids, which are characterized by a diphenylpropane (C6 C3 C6) skeleton. Quercetin is among the branches of flavanols that are characterized by a hydroxyl compound at 3 carbon as well as a linked between carbon 2 and 3<sup>11</sup>. Quercetin shape is changeable based on the temperature and location for instance it is a solid at room temperature which has a yellow color in the visible spectrum and two strong absorbance bands in the ultraviolet<sup>12</sup>.

Quercetin is found in many members of the plant kingdom, including many edible species<sup>13</sup>. Quercetin is found in high levels in onion, lettuce, broccoli, cranberries and apple skins<sup>11</sup>. Quercetin along with other flavonoids is also found in a number of common beverage including tea, beer and wine. Quercetin is most widely noticed in plants, and ingested, as a glycoside with the linkage to the sugar happens through the hydroxyl group at the C3 position to form quercetin-3-O-glycoside<sup>13</sup>.

Treatment of inflammation is a long discussion as the conventional NSAIDS are well known to cause Adverse Drug Reactions. Moreover there is undertaking study to promote safer and more effective drugs for the therapy of inflammation. In view of this and on account of assumed worthlessness of this compound in the traditional treatment. Hence the current investigation was done to assess the anti-inflammatory process of Quercetin in experimental animal sample.

## MATERIAL AND METHODS

### Chemicals

Flavonoids (Quercetin), Carrageenan, Indomethacin were purchased from Sigma

Aldrich Co. (St Louis, MO, USA). Polysorbates 80 USP (Tween 80) as a suspending agent for Indomethacin, Carboxymethyl cellulose 5% as a suspending agent for flavonoids were purchased from Himedia Chemical Co. India.

### Animals

Healthy adult male guinea pigs weighing between (350 ± 650 g) were obtained from the animal house of Biology Department, Ibb University-Yemen and kept for 1 week on a commercial diet in environmentally controlled conditions (25 ± 5°C, 55 ± 5% humidity and 12h light–dark cycle) to acclimatize with free access to diet and water *ad libitum*. Animals were fasted overnight and weighed before the experiment. The study protocol was approved by Institutional Animal Ethics Committee.

### Methodology<sup>14</sup>

Animals were randomly divided into 3 groups of 6 pigs each; group I: Control (1ml of 5% carboxymethyl cellulose suspension); group II: Standard drug (indomethacin 10 mg/kg); group III: Test drug (Quercetin 20 mg/kg). All the doses were administered orally. The *in vivo* anti-inflammatory activity was studied using carrageenan induced paw edema.

### Carrageenan induced rat paw edema model<sup>14</sup>

0.1 ml of 1% Carrageenan was injected aseptically into the sub plantar surface of right hind paw of each pig. Paw edema was measured by mercury plethysmograph (VGO Basile, Italy) at '0' hour and at the end of '4' hours. The difference between the 0 and 4<sup>th</sup> hour reading gives the actual edema. Percentage inhibition (protection) against edema formation was taken as an index of acute anti-inflammatory activity. It was calculated by:

Percentage inhibition of inflammation =  $[(V_c - V_t) / V_c] \times 100$ .

Where:  $V_c$  = mean paw edema volume in the control group.

$V_t$  = mean paw edema volume in the drug-treated group.

#### Cotton pellet-induced granuloma<sup>14</sup>

The cotton pellet induced granuloma in pigs was studied. The animals were divided into three groups of six animals in each group. Cotton pellets weighing  $50 \pm 1$  mg were autoclaved and implanted subcutaneously into both sides of the groin region of each pig. The first group served as control and received the vehicle only (carboxymethyl cellulose 5%). Second group of animals was administered with standard drug indomethacin (10 mg/kg). The animals of third group were treated with Quercetin (20 mg/kg). All the doses were administered orally. On the 8<sup>th</sup> day, the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C weighed and compared with control.

#### Statistical analysis

The effects of different drugs under study was presented by calculating the mean and SD of the outcome parameters. One way Analysis of Variance (ANOVA) and independent samples T test was applied to see the difference between any two groups at a time (5% significance level). SPSS for windows (version 15) was applied in the statistical analysis.

## RESULTS AND DISCUSSION

### Anti-inflammatory studies

#### Carrageenan induced rat paw edema

The anti-inflammatory effect of the Quercetin using carrageenan induced edema tests is expressed in (Table I). In this test, the positive control (Indomethacin 10 mg/kg)

significantly ( $P < 0.05$ ) decreased the paw edema by 65%, 71%, 72% and 74% after 1, 2, 3 and 4 hours compared with non-treated control group value. Pigs with the Quercetin at 20 mg/kg/body weight significantly decreased ( $P < 0.05$ ) the carrageenan induced edema paw volume edema by 34%, 49%, 61% and 68 % after 1, 2, 3 and 4 hours respectively compared with non-treated control group values.

#### Cotton pellet induced granuloma in rats

The results of anti-inflammatory activity of Quercetin in cotton pellet induced granulomas shown in Table II. Quercetin (20 mg/kg) showed decreased significantly ( $P < 0.05$ ) in granuloma formation as compared to control group. The percent of inhibition were significantly 0%, 37%, and 34% for non-treated control, (10 mg/kg) indomethacin and (20 mg/kg) Quercetin, respectively (Graph 3).

## DISCUSSION

The anti-inflammatory process of flavonoids (Quercetin) was assessed employing acute (carrageenan-induced paw edema) and chronic (cotton pellet granuloma) models of inflammation. The current study discovered that Quercetin at a dose of 20 mg/kg have significant anti-inflammatory process in experimental animals.

Carrageenan-induced paw edema as *in vivo* model of inflammation was picked to evaluate the anti-inflammatory activity of natural products, especially in the acute phase of inflammation<sup>15</sup>. Carrageenan-induced inflammation is a biphasic phenomenon. The first phase, which occurs between 0 to 2.5 hours of injection of the phlogistic agent, has been attributed or serotonin. The second phase of inflammatory reaction which is measured at 3 hour is formed by the release of bradykinin, protease, prostaglandin and

lysosome<sup>16</sup>. The results revealed that administration of Quercetin compound inhibited the edema starting from the first hour and during all phases of inflammation, which is perhaps inhibition of various phenomena and chemical mediators of inflammation. The cotton pellet granuloma way has been largely employed to assess the increasing phase of chronic inflammatory reaction. The inflammatory granuloma is a typical feature of reaction. The events involved in this phase of inflammation are proliferation of macrophages, neutrophils and fibroblasts. The dry weight granuloma formed correlates with the amount of granulomatous tissue formed<sup>17</sup>. It can be inferred from table II the Quercetin 20 mg/kg significantly inhibited the granuloma tissue formation. This suggests the Quercetin have inhibitory effect on the proliferative phase of inflammation and probably may act by inhibition. This impact may be due to the cellular migration to injured sites and accumulation of collagen. Cell migration appears as a result of much different process including adhesion and cell mobility. Flavonoids are naturally occurring compounds containing in vascular plants. Such compounds have been noticed to have anti-inflammatory features, both *in vitro* and *in vivo*<sup>18</sup>. A Several flavonoids have been found out to have significant anti-inflammatory activity<sup>19</sup>. This study emphasized that, the flavonoids in charge of its anti-inflammatory activity.

Non-steroidal anti-inflammatory drugs (NSAID) for instance indomethacin employed in this study are proved to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation mediating agent prostaglandin E2 (PGE2) from arachidonic acid<sup>20</sup>. Therefore, the pattern of anti-inflammatory activity exhibited by this extract was similar to that of indomethacin. (See table 1&2 and graph 1-3.)

## CONCLUSION

Based on the aforementioned results it may can be concluded that Quercetin show huge well significant ( $P < 0.05$ ) anti-inflammatory activity in comparison to indomethacin against carrageenan induced paw edema and cotton pellet granuloma mode. It is used as an adjuvant linked with factors that fight serious inflammation and decrease portion and minimal adverse impacts for the drug of inflammation because of its simple existence efficiency security and powerfulness. Therefore Quercetin (20 mg/kg) shows the moderate activity in carrageenan induced paw edema model. This study sheds light on the efficacy of Quercetin as an anti-inflammatory factor.

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**Table 1.** Effect of Quercetin and Indomethacin on carrageenan induced edema paw volume in male guinea pig

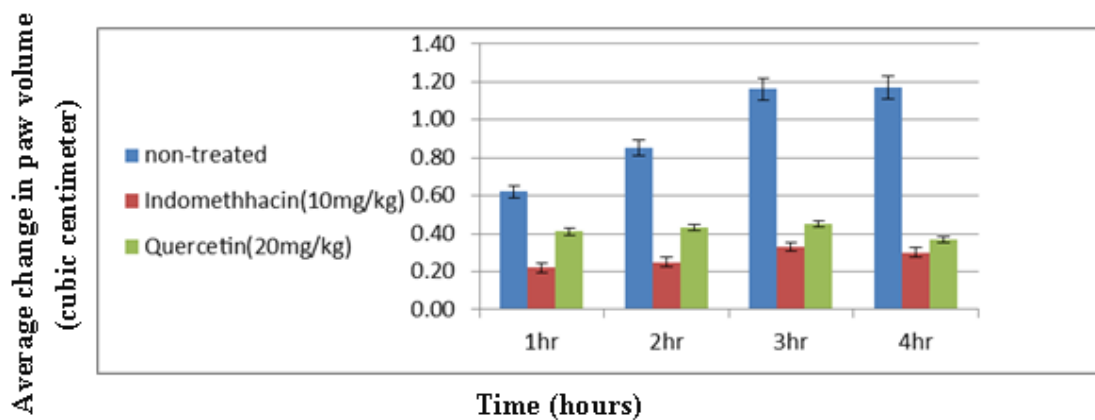
Groups	Mean paw edema (cm)± SD				%Percent inhibition at 4 hours
	1hr.	2hr.	3hr.	4hr.	
Non-treated control	0.62 ±0.009	0.85 ±0.005	1.16±0.005	1.17 ±0.01	0.0%
Indomethacin (10mg /kg)	0.22 ±0.009*	0.25 ±0.005*	0.33 ± 0.005*	0.30 ±0.01*	74%
Quercetin (20mg/ kg)	0.41 ±0.009*	0.43 ±0.005*	0.45 ± 0.005*	0.37 ±0.01*	68%

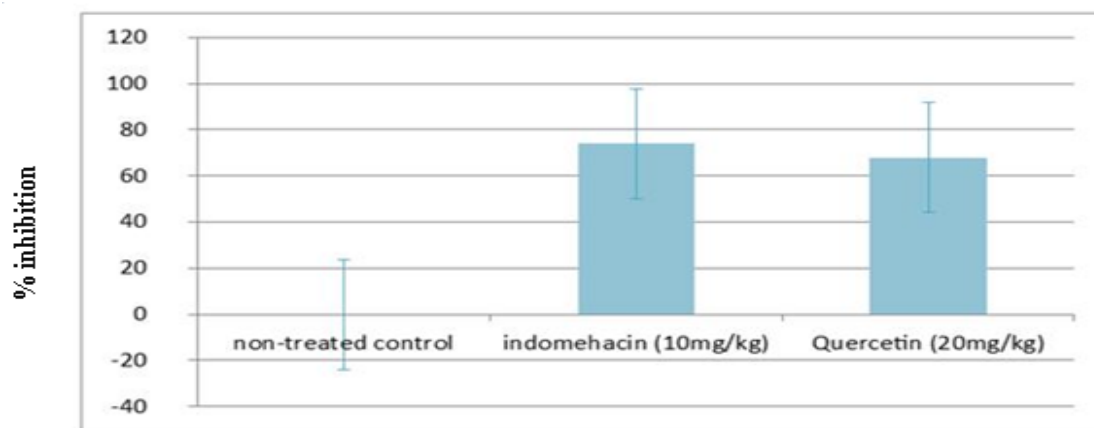
Values are expressed as mean ± SD, n=6 animals per group, \*P<0.05ascompared to control (one-way ANOVA).

**Table 2.** Anti-inflammatory activity of Quercetin and reference standard drug Indomethacin (10mg kg) on cotton pellet granuloma in normal male guinea pigs

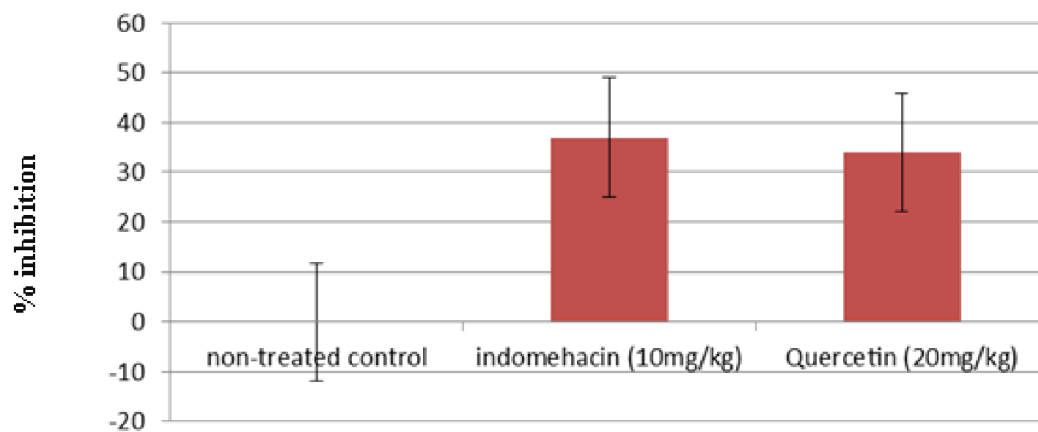
Treatment	Weight of dry granuloma (mg)	% of inhibition
Non-treated control	86.67 ± 5.41	0%
Indomethacin (10mg/kg)	55.00 ± 1.95*	37%
Quercetin( 20 mg/kg)	57.33 ± 1.31*	34%

Results are Mean ± SD (n=6) \*P<0.05 Compared to Control.

**Graph 1.** Effect of Quercetin and Indomethacin on carrageenan-induced edema paw volume in male guinea pig



**Graph 2.** Percent inhibition (%) with the Control, Standard (indomethacin) and the test compound (Quercetin), in the carrageenan induced paw edema



**Graph 3.** Anti-inflammatory activity of Quercetin and reference standard drug Indomethacin (10mg/kg) on cotton pellet granuloma in normal male guinea pigs