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ORIGINAL ARTICLE

Investigation of Analgesic and Anti-inflammatory Activities and Dose Optimization of Quercetin and Its Few Novel Semisynthetic O-methylated Derivatives

Abhijit Bose[*1], Sankhadip Bose[*2], Kunal Gupta[3], Purnendu Kumar Roychoudhury[1], Kamalika Mazumder[4], Subhasis Maity[5], Sabyasachi Banerjee[6], Sujayaita Mazumder[2]

[1]*T. C. G. Life Sciences Pvt. Limited (Chembiotek), Plot No-7, Salt Lake Electronics Complex, BN Block, Sector V, Kolkata, West Bengal-700091, India*

[2]*School of Pharmacy, The Neotia University, Sarisa, Diamond Harbour Road, South 24 Parganas, West Bengal 743368, India*

[3]*Eminent college of Pharmaceutical Technology, Barasat, Kolkata, West Bengal-700126, India*

[4]*BCDA College of Pharmacy & Technology, Hridaypur, Barasat-700127, India*

[5]*NSHM Knowledge Campus, Kolkata – Group of Institutions, 124 B.L. Saha Road, Kolkata, West Bengal-700053, India*

[6]*Department of Pharmaceutical Chemistry, Gupta College of Technological Sciences, Asansol 713 301, West Bengal, India*

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ABSTRACT: Pain and inflammation are associated with numerous disorders, and many conventional treatments carry unwanted side effects. Plant-derived compounds like flavonoids play a significant role in alleviating such conditions by scavenging free radicals, chelating metal ions, or inhibiting radical-producing proteins. This study aimed to perform a preclinical pilot investigation of quercetin and its semisynthetic O-methylated derivatives to optimize their dose and establish their analgesic and anti-inflammatory efficacy using male Albino Wistar rats. For pilot study, the doses were selected according to OECD guideline 423. According to OECD guideline 423, doses of 0.025, 0.05, 0.1, and 0.2 g kg^{-1} were selected. Analgesic activity was assessed using the tail-flick method, while anti-inflammatory activity was evaluated with the Carrageenan-induced paw edema test. Diclofenac sodium (0.01 g kg^{-1}) was used as the reference drug. Quercetin and its derivatives were tested at two optimized doses of 0.1 g kg^{-1} and 0.2 g kg^{-1}. From the current pilot study, it was revealed that the selected standardized two doses (0.1 g per kilogram and 0.2 g per kilogram) of quercetin, its semisynthetic derivatives possessed significant activity in a dose-related pattern. The aforementioned approaches were conducted under a controlled and closely monitored environment, according to rigorous protocols for the ethical treatment of experimental animals, and with the explicit approval of the IAEC. The current investigation concluded that quercetin and its semisynthetic *O*-methylated derivatives had potent analgesic and anti-inflammatory effects when administered a dosage of 0.1 g per kilogram and 0.2 g per kilogram.

KEYWORDS

Quercetin;
O-methylated quercetin derivatives;
Pilot study;
Analgesic;
Anti-inflammation;
Toxicity

*Corresponding author: boseabhiphtk@gmail.com; sankha.bose@gmail.com (A. Bose; S. Bose)
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INTRODUCTION

There exist various types of illnesses, ailments, and conditions within the human body. Among these, inflammation and pain are the most severe forms of discomfort [1], Pain is a commonly experienced sensory and emotional phenomenon that is typically associated with the potential for significant tissue damage resulting from various stimuli [2]. Pain is commonly acknowledged as the primary motivator for individuals seeking medical attention [3], arises because of the stimulation of nociceptors, which are pain receptors [4]. Analgesics refer to pharmaceutical substances employed for alleviating various forms of pain. The analgesic agent functions by interacting with pain receptors, specifically the Mu, Kappa, and Delta receptors [5]. Inflammation can be described as a common physiological response to tissue damage in the human body, resulting from physical injury as well as exposure to harmful chemical substances or microbiological agents that cause harm to cells and tissues [6]. The phenomenon of inflammation is mostly linked to the synthesis and secretion of cytokines, including IL-6, TNF-α, and IL1β, by cells that have been stimulated. Cytokines are known to have a substantial influence on the host's defense systems [7]. The management of inflammatory infections in medical facilities is dependent on the administration of medications that fall into either the steroidal or the non-steroidal synthetic therapeutic categories. However, drugs may sometimes result in significant adverse effects when used over extended periods [8]. Pyrexia, also known as fever, can arise from various factors such as contamination, tissue injury, inflammation, joint displacement, or exposure to hazards. Additionally, pathogenic microorganisms like bacteria or viruses can trigger the body's immune response, leading to the development of fever [9]. Fever is commonly observed as a secondary consequence of infection, exposure to pathogens, or other pathological conditions [10].

Analgesic and corticosteroids, which is frequently be employed for contemporary medical practice in mitigate of inflammation of pain, primarily offer symptomatic alleviation. The utilization of these medications over an extended period has been linked to significant detrimental consequences. Therefore, the pursuit of a novel and secure analgesic and anti-inflammatory medication remains in progress [11].

Traditional medicines, particularly those derived from herbs, are widely recognized as a primary reservoir of various therapeutic compounds [12]. Additionally, they function as a catalyst for the creation of novel compounds that exhibit improved pharmacological characteristics [13, 14]. Several herbal medicines continue to be utilized in medical practice without significant adverse effects [15]. Therefore, it is imperative to explore new pharmaceutical compounds that exhibit enhanced efficacy and cost efficiency.

Within this domain, flavonoids, specifically quercetin, have gained recognition for their diverse array of functionalities. This study emphasizes the effectiveness of the compound in inflammation and pain. The investigation offers a promising avenue for exploring the potential of this natural compound and its semi-synthetic derivatives as remedies for pain relief and inflammation. Moreover, this investigation has the potential to establish a standard for future examinations or the observed phenomena.

MATERIALS AND METHODS

Drugs and chemicals

All the substances used in this investigation are of laboratory grade. We bought carrageenan from Hi Media in Mumbai, India. Sodium salt of diclofenac was procured from Macleods Pharmaceuticals Ltd. (Mumbai, Maharashtra, India). The quercitrin as well as its two derivatives namely 3,7-di-*O*-methylquercetin and 3,7,4′-tri-*O*-methylquercetin were taken from our earlier experiments.

Experimental animals

The present study used Wistar albino rats of both genders, with a weight range of 180 g to 200 g. The experimental methodology used in the present investigation adhered to the rules set out through CPCSEA. Furthermore, permission given by the IAEC under the reference number NCPT/IAEC-008/2022.

Toxicity Study

Acute toxicity research was conducted on Quercetin and its O-methylated derivatives, following the standards provided *via* OECD 423. The investigation was performed on female *Mus musculus* with a body weight of 25-30 g. To assess acute toxicity, observations were conducted at various time intervals including 0 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, and 12 hr, followed by daily observations for 14 days to detect any indications of toxicity. The animals exhibited no indications of toxicity when administered a maximum dose of 2 g kg^{-1} body weight during the conducted experiment. These findings demonstrate the safety of oral administration of the quercetin and O-methylated quercetin derivatives.

The toxicity study employed two dosages of 0.3 g per kilogram and 0.2 g per kilogram administered relative to body weight. According to the OECD 423 guideline, quercetin and its different O-methyl derivatives do not exhibit any toxicity or death when provided up to a dosage of 0.3 g per kilogram and 0.2 g per kilogram weight of the body, respectively, in our experiment on acute toxicity. For the pilot study, we choose four dosages of 0.025, 0.05, 0.1, and 0.2 mg kg^{-1} body weight as a trial-and-error methods to standardize and optimize the lower and higher doses for optimum biological activities.

Experimental Design

Assessment of analgesic activity

The study used adult male Wister albino rats weighing between 180 and 200 grams, were procured from the central animal home at the NSHM campus of Pharmaceutical Technology. The animal was housed in a controlled laboratory environment, maintained at ambient temperature and with typical levels of humidity. The participants were provided with a standardized diet and unrestricted access to water. The animals were carefully chosen and categorized into six unique groups, with a total sample size of six (n=6). For each group that is control, standard, three different test drugs respectively Quercetin, 3,7-di-*O*-methyl-quercetin, and 3,7,4′ -tri-*O*-methyl-quercetin with four different concentrations (0.025 gram per kilogram, 0.05 gram per kilogram, 0.1 gram per kilogram, 0.2 gram per kilogram) were used in the pilot study to optimize the efficacy.

Tail-flick method

Before assessing the analgesic activity, the animals underwent a sensory evaluation by gently immersing the distal end of their tails (5 cm) in warm water (55 °C). Within a brief temporal interval, the animals promptly respond by retracting their tails from the heated water. The measurement of the reaction period's duration was conducted using a stopwatch. The assessment of reaction time occurred after the delivery of the component of the medicine [16]. Animals (Adult male Wister albino rats, 180–200 g) were chosen, followed by division into six distinct groups for every drug, with each division consisting of six animals. The animals belonging to Group A (control) were subjected to oral administration of a 0.5% carboxymethyl cellulose (CMC) solution at a dose of 10 milligrams per animal. Rats belonging to Group B (standard) were subjected to 10 milligrams of diclofenac sodium given orally.

Animals in Groups C1, C2, C3, and C4 were given test drug quercetin orally, doses of 0.025 gram per kilogram, 0.05 gram per kilogram, 0.1 gram per kilogram, 0.2 gram per kilogram respectively. Animals in Group D1, D2, D3, and D4 were given test drug 3,7-di-*O*-methylquercetin orally at the dose of 0.025 gram per kilogram, 0.05 gram per kilogram, 0.1 gram per kilogram, 0.2 gram per kilogram respectively. Animals in E1, E2, E3, and E4 were administered test drug 3,7,4′ -tri-*O*-methylquercetin orally at the dose of 0.025 gram per kilogram, 0.05 gram per kilogram, 0.1 gram per kilogram, 0.2 gram per kilogram respectively. The reaction is recorded at 60 minutes and 120 minutes after the drug administration [16].

Evaluation of the anti-inflammatory effect

Carrageenan-loaded paw edema method

The study used adult male Wister albino rats weighing between 180-200 grams, with a total sample size of 6. The paw edema model treated with carrageenan was chosen for analysis. Quercetin, 3,7-di-*O*-methyl-

quercetin, and 3,7,4′-tri-*O*-methyl-quercetin were the three medications evaluated for their anti-inflammatory properties [17] in the experimental study using the Carrageenan-induced paw edema model. The rat paw edema test, which is induced by carrageenan, has been used to determine anti-inflammatory activity [18]. A sub-plantar injection of 100 µL of freshly made 1% carrageenan solution in distilled water was given to the experimental subjects, causing edema in their right hind paws. All groups, except for group A, completed this process.

For each medicine, a total of seven groups including each six animals were created from the selected animals.

The animals belonging to Group A (Control) were subjected to oral administration of a 0.5% CMC solution, amount 10 milligram per kilogram. Animals in Group B (negative control) received a subcutaneous dose of 0.1 milliliter of a 1% weight/volume carrageenan solution loaded normal saline as negative control. Animals belonging to Group C (Standard) were subjected to orally deliver of diclofenac sodium dose 10 milligram per kilogram.

Animals in Groups D1, D2, D3, and D4 were given test drug quercetin orally delivering 0.025 g per kilogram, 0.05 g per kilogram, 0.1 g per kilogram, 0.2 g per kilogram. Group of a animals noted as E1, E2, E3, E4 were given test drug 3,7-di-*O*-methyl-quercetin orally at the dose of 0.025 gram per kilogram, 0.05 gram per kilogram, 0.1 gram per kilogram, 0.2 gram per kilogram respectively. Group of animals noted as F1, F2, F3, and F4 were administered test drug 3,7,4′-tri-*O*-methyl-quercetin orally at the dose of 0.025 gram per kilogram, 0.05 gram per kilogram, 0.1 gram per kilogram, 0.2 gram per kilogram respectively.

Carrageenan solution, with a concentration of 1% weight/volume and a volume of 0.1 ml, administered through injection into the sub-plantar tissue of each rat's right hind paw, except those in group A, after 30 minutes period of drug delivery. The paw volumes of the animals were taken at regular intervals of 1, 2, and 3 hours utilizing a plethysmometer. Additionally, the extent of swelling was documented. The data were presented as a percentage representing the degree of swelling relative to the initial volume of the hind paw for each animal.

Statistical analysis

The mean ± the standard error of the mean (SEM) has been used to express each value. Using Graph Pad Prism software, a one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test were used to conduct the statistical analysis of the results.

RESULTS

Acute toxicity Study

No deaths were observed in relation to the administration of quercetin and its O-methylated derivatives was taken 2 gram per kilogram. Following administration of a solitary dose, the animals were subjected to a 14-day observation period. No detrimental indications were detected in the subjects of the experiment.

Assessment of analgesic activity for quercetin and its *derivatives*

Tail-flick test

Effects of Quercetin and its O-methylated Derivatives on tail-flick method

Based on our investigation, the lowest dose at which the animals exhibited a statistically significant result (P value less than 0.001) was determined to be 0.1 g per kilogram. This dose was designated as the low dose, while the subsequent dosage of 0.2 g per kilogram, which is double the lowest dosage was designated as the high dose. The progression of doses was calculated using a geometric ratio of ½, whereby each subsequent dose is double the previous dose. The reference drug used in the study was diclofenac sodium dose 0.01 g per kilogram administered orally. The administration of diclofenac sodium at a dosage of 0.01 g kg^{-1} orally produces extremely rise in nociceptive reaction time at both the 60-minute and 120-minute time points. In the tail flick immersion test, it was observed that quercetin and its O-methylated derivatives, specifically 3,7-di-methyl-quercetin and 3,7,4′-tri-*O*-methyl-quercetin, demonstrated a notable elevation in reaction time to thermal stimuli, indicating the manifestation of pain-related effects.

Quercetin (Test Drug-1) (Figure 1) 200 mg kg^{-1} (group-C4) animals show result [P < 0.001] rise in tail flick response time at 60 minutes interval [11±0.59 sec] and at 120 minutes interval [12±0.28 sec] when compared with normal control animals at 60 minutes interval [3±0.29 sec] and at 120 minutes interval [3±0.31 sec] respectively. Quercetin 100 mg kg^{-1} (group-C3) animals show a result [P < 0.001] rise in tail flick response time at 60 minutes interval [10±0.35 sec] and at 120 minutes interval [10±0.91 sec] when compared with normal control animals at 60 minutes interval [3±0.29 sec] and at 120 minutes interval [3±0.31 sec] respectively. Quercetin was given at the dose of 50 mg kg^{-1} to the group-C2 animals. The animals failed to show significant results when compared with normal control animals at 60-minute intervals [3±0.29 sec] and at 120-minute intervals [3±0.31sec] respectively. After administering quercetin 25 mg kg^{-1} (group-C1) animals failed to show significant results when compared with normal control animals at 60-minute intervals [3±0.29 sec] and at 120-minute intervals [3±0.31 sec] respectively. So, quercetin demonstrates notable effects its dosage levels of 0.1 gram per kilogram and 0.2 gram per kilogram, administered orally, after 60 and 120 minutes. All the values are shown in Table 1.

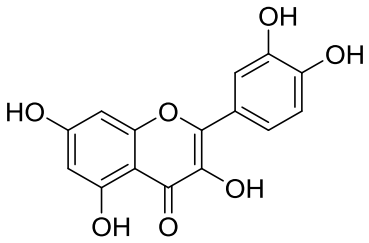

Figure 1. Quercetin

3,7-di-*O*-methyl-quercetin (Test Drug-2) (Figure 2) 200 mg kg^{-1} (group-D4) animals show result [P < 0.001] rise in tail flick response time at 60 minutes interval [13±0.36 sec] and at 120 minutes interval [13±0.29 sec] when compared with normal control animals at 60 minutes interval [2±0.13 sec] and at 120 minutes interval [3±0.72 sec] respectively. 3,7-di-*O*-methylquercetin on 100 mg kg^{-1} (group-D3) animals showed result [P < 0.001] rise in tail flick response time at 60 minutes interval [12±0.62 sec] and at 120 minutes interval [12±0.17 sec] respectively. 3,7-di-*O*-methyl-quercetinon 50 mg kg^{-1} (group-D2) animals failed to show significant results when compared with normal control animals at 60 minute intervals [4±0.83 sec] and at 120 minute intervals [4±0.45 sec] respectively. 3,7-di-*O*-methylquercetin on 25 mg kg^{-1} (group-D1) animals failed to show significant results when compared with normal control animals at 60 minutes interval [3±0.39 sec] and at 120 minutes interval [3±0.41 sec] respectively. All the values are shown in Table 2.

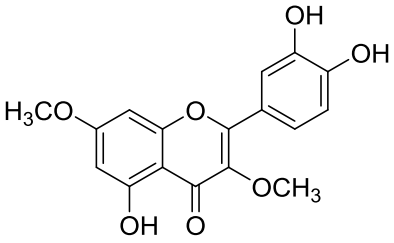

Figure 2. 3, 7-di-*O*-methyl quercetin

3,7,4′-tri-*O*-methylquercetin (Test Drug-3) (Figure 3) 200 mg kg^{-1} (group-E4) animals showed a result [P < 0.001] rise in tail flick response time at 60 minutes interval [12±0.56 sec] and at 120 minutes interval [13±0.95 sec] when compared with normal control animals at 60 minutes interval [2±0.14 sec] and at 120 minutes interval [2±0.42 sec] respectively. 3,7,4′-tri-*O*-methylquercetin 100 mg kg^{-1} (group-E3) animals showed a result [P < 0.001] rise in tail flick response time at 60 minutes intervals [11±0.23 sec] and at 120 minutes

intervals [12±0.87 sec] respectively. 3,7,4′-tri-*O*-methylquercetin 50 mg kg^{-1} (group-E2) animals failed to show significant results when compared with normal control animals at 60 minutes interval [3±0.36 sec] and at 120 minutes intervals [3±0.23 sec] respectively. 3,7,4′-tri-*O*-methylquercetin 25 mg kg^{-1} (group-E1) animals failed to show significant results when compared with normal control animals at 60 minute intervals [3±0.82 sec] and at 120 minute intervals [2±0.79 sec] respectively (Table 3).

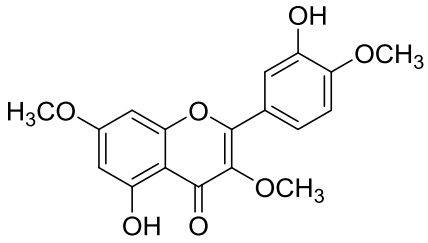

Figure 3. 3, 7, 4´-tri-O-methyl quercetin

In contrast, noteworthy effects were observed with *O*-methylated derivatives, specifically 3,7-di-methyl-quercetin and 3,7,4′-tri-*O*-methyl-quercetin, after 60 min and 120-minutes dose of 0.1 gram to kilogram and 0.2 gram to kilogram administered orally. Quercetin and its O-methylated derivatives, taken orally dosage 0.025 gram per kilogram and 0.050 gram per kilogram, did not showed result in statistically significant effects (Tables 1-3).

Assessment of anti-inflammatory *activity for Quercetin and its* derivatives

Carrageenan-loaded paw edema test

Effects of quercetin and its o-methylated Quercetin Derivatives on Carrageenan-induced paw edema:

The Carrageenan-loaded rat paw edema model demonstrates the anti-inflammation properties of quercetin and its *O*-methylated derivatives, as well as diclofenac sodium. Giving of medication, diclofenac sodium at a dose of 0.01 g per kilogram orally demonstrated more pronounced effects in reducing carrageenan-induced inflammation at 2- and 3-hours post-administration.

According to our investigation, the lowest dose at which the animals exhibited a statistically significant result ($P<0.001$) was determined to be 100 mg kg^{-1}. This dose was designated as the low dose, while the dosage of 0.2 g per kilogram, i.e. twice the lowest dose, was designated as the high dose. The progression of doses was calculated using a geometric ratio of ½, whereby each subsequent dose was double the previous dose. The reference drug used in the study was diclofenac sodium at a dosage of 0.01 g per kilogram giving the medication orally. Administration of quercetin and its O-methylated derivatives at doses of 0.025 g per kilogram and 0.05 g per kilogram via oral route did not result in any statistically significant effects. Quercetin, 3,7-di-methylquercetin and 3,7,4′-tri-*O*-methylquercetin at the dose of 0.1 g per kilogram and 0.2 g per kilogram displayed maximum reduction of paw volume after 2h and 3h (Table 4-6 respectively).

Table 1. Impact of quercetin on the tail-flick technique.

Name of the therapy group and dose amount	Number of Animals	Response time in seconds after the drug has been administered	
		60 min	120 min
Control (carboxymethyl cellulose) 0.5% (group-A)	six	3±0.29	3±0.31
Standard Diclofenac sodium 10 mg kg^{-1} (group-B)	six	13±0.52***	13±0.64***
Quercetin Test Drug-1 25 mg kg^{-1} (group-C1)	six	4±0.62	4±0.81
Quercetin Test Drug-1 50 mg kg^{-1} (group-C2)	six	4±0.79	5±0.26
Quercetin Test Drug-1 100 mg kg^{-1} (group-C3)	six	10±0.35***	10±0.91***
Quercetin Test Drug-1 200 mg kg^{-1} (group-C4)	six	11±0.59***	12±0.28***

For Dunnett's test (n = 6), one-way ANOVA was used, and all data were presented as Mean ±S.E.M. ***(P<0.001) in contrast to the normal group.

Table 2. Results of 3,7-di-*O*-methylquercetin on tail-flick method.

Name of the therapy group and dose amount	Number of Animals	Response time in (sec) after drug administration.	
		60 min.	120 min
Control 0.5% carboxymethyl cellulose (CMC) (group-A)	six	2±0.13	3±0.72
Standard Diclofenac sodium 10 mg kg^{-1} (group-B)	six	14±0.81***	14±0.63***
3,7-di-methylquercetin Test Drug-2 25 mg kg^{-1} (group-D1)	six	3±0.39	3±0.41
3,7-di-methylquercetin Test Drug-2 50 mg kg^{-1} (group-D2)	six	4±0.83	4±0.45
3,7-di-methylquercetin Test Drug-2 100 mg kg^{-1} (group-D3)	six	12±0.62***	12±0.17***
3,7-di-methylquercetin Test Drug-2 200 mg kg^{-1} (group-D4)	six	13±0.36***	13±0.29***

For Dunnett's test (n = 6), one-way ANOVA was used, and all data were presented as Mean ±S.E.M. ***(P<0.001) in contrast to the normal group.

Table 3. Results of 3,7,4′-tri-*O*-methylquercetin on tail-flick method

Name of the therapy group and dose amount	Number of Animals	Response time in seconds after the drug has been administered	
		60 min	120 min
Control **0.5% carboxymethyl cellulose (group-A)**	six	2±0.14	2±0.42
Standard **Diclofenac sodium** **10 mg kg^{-1} (group-B)**	six	13±0.36***	14±0.51***
3,7,4′-tri-*O*-methylquercetin **Test Drug-3** **25 mg kg^{-1} (group-E1)**	six	3±0.82	2±0.79
3,7,4′-tri-*O*-methylquercetin **Test Drug-3** **50 mg kg^{-1} (group-E2)**	six	3±0.36	3±0.23
3,7,4′-tri-*O*-methylquercetin **Test Drug-3** **100 mg kg^{-1} (group-E3)**	six	11±0.23***	12±0.87***
3,7,4′-tri-*O*-methylquercetin **Test Drug-3** **200 mg kg^{-1} (group-E4)**	six	12±0.56***	13±0.95***

For Dunnett's test (n = 6), one-way ANOVA was used, and all data were presented as Mean ±S.E.M. ***(P<0.001) in contrast to the normal group.

Table 4. Reduction of paw volume by Quercetin on carrageenan-loaded paw edema test

Name of the therapy group and dose amount	Paw volume in ml hr^{-1}				
	Number of Animals	Initial volume	1 hour	2 hour	3 hour
Control **0.5% carboxymethyl cellulose (group-A)**	six	2.3±0.25	2.3±0.83	2.3±0.21	2.3±0.38
Diseased Negative Control (only carrageenan) 0.1 ml (1%w/v) (group-B)	six	2.4±0.38	6.8±0.84$^{###}$	6.9±0.25$^{###}$	6.9±0.65$^{###}$
Standard **Diclofenac sodium** **10 mg kg^{-1} (group-C)**	six	2.3±0.19	4.1±0.43**	3.6±0.30***	2.1±0.17***
Quercetin (Test drug-1) **25 mg kg^{-1} (group-D1)**	six	2.4±0.21	6.5±0.49	6.4±0.87	6.1±0.79
Quercetin (Test drug-1) **50 mg kg^{-1} (group-D2)**	six	2.4±0.94	5.9±0.68	5.7±0.21	5.4±0.41
Quercetin (Test drug-1) **100 mg kg^{-1} (group-D3)**	six	2.3±0.39	4.3±0.21**	4.1±0.39**	4.0±0.42**
Quercetin (Test drug-1) **200 mg kg^{-1} (group-D4)**	six	2.4±0.78	3.4±0.83***	3.3±0.59***	3.1±0.39***

Each value is presented as Mean±SEM. N=6. One-way ANOVA was used in the test, and Tukey's Multiple Comparison Test was then performed. ### (P<0.001) in contrast to the control group. **(P<0.01) in comparison to the diseased negative control group; ***(P<0.001) in comparison to the diseased negative control group.

Table 5. Reduction in paw volume by 3,7-di-*O*-methylquercetin on carrageenan-loaded paw edema test

Name of the therapy group and dose amount	Paw volume in ml hr^{-1}				
	Number of Animals	Initial volume	1 hour	2 hour	3 hour
Control **0.5% carboxymethyl cellulose** **(group-A)**	six	2.4±0.59	2.4±0.97	2.4±0.81	2.4±0.29
Diseased Negative Control **(only carrageenan)** **0.1 ml (1%w/v)** **(group-B)**	six	2.3±0.53	6.7±0.30###	6.8±0.13###	6.8±0.24###
Standard **Diclofenac sodium** **10 mg kg^{-1} (group-C)**	six	2.4±0.59	4.2±0.98**	3.4±0.25***	2.0±0.17***
3,7-di-*O*-methylquercetin **(Test drug-2)** **25 mg kg^{-1} (group-E1)**	six	2.2±0.31	6.6±0.67	6.3±0.82	6.3±0.58
3,7-di-*O*-methylquercetin **(Test drug-2)** **50 mg kg^{-1} (group-E2)**	six	2.2±0.78	6.5±0.72	6.3±0.82	6.1±0.54
3,7-di-*O*-methylquercetin **(Test drug-2)** **100 mg kg^{-1} (group-E3)**	six	2.4±0.64	4.2±0.78**	3.8±0.29**	3.7±0.81**
3, 7-di-*O*-methylquercetin **(Test drug-2)** **200 mg kg^{-1} (group-E4)**	six	2.3±0.39	3.5±0.54***	3.5±0.19***	3.2±0.24***

Each value is presented as Mean±SEM. N=6. One-way ANOVA was used in the test, and Tukey's Multiple Comparison Test was then performed. ### (P<0.001) in contrast to the control group. **(P<0.01) in comparison to the diseased negative control group; ***(P<0.001) in comparison to the diseased negative control group.

Table 6. Reduction in paw volume by 3,7,4′-tri-*O*-methylquercetin on carrageenan-loaded paw edema test.

Name of the therapy group and dose amount	Paw volume in ml h^{-1}				
	Number of Animals	Initial volume	1 hr	2 hr	3 hr
Control **0.5% carboxymethyl cellulose** **(group-A)**	six	2.4±0.63	2.4±0.48	2.4±0.21	2.4±0.95
Diseased Negative Control **(only carrageenan)** **0.1 ml (1% w/v)** **(group-B)**	six	2.4±0.61	6.9±0.55###	7.0±0.17###	6.9±0.21###
Standard **Diclofenac sodium** **10 mg kg^{-1} (group-C)**	six	2.3±0.17	3.1±0.78***	2.8±0.19***	2.5±0.41***

3, 7, 4′-tri-*O*-methylquercetin (Test drug-3) 25 mg kg^{-1} (group-F1)	six	2.3±0.63	6.6±0.86	6.6±0.35	6.2±0.32
3,7,4′-tri-*O*-methylquercetin (Test drug-3) 50 mg kg^{-1} (group-F2)	six	2.4±0.18	6.7±0.39	6.3±0.16	6.2±0.29
3, 7, 4′-tri-*O*-methylquercetin (Test drug-3) 100 mg kg^{-1} (group-F3)	six	2.3±0.27	4.9±0.18**	4.9±0.35**	4.6±0.76**
3,7,4′-tri-*O*-methylquercetin (Test drug-3) 200 mg kg^{-1} (group-F4)	six	2.3±0.32	4.5±0.18**	4.2±0.32**	4.0±0.48**

All values are expressed as MEAN±SEM. N=6. Test employed One-way ANOVA followed by Tukey's Multiple Comparison Test. ###(P<0.001) when compared to normal group. **(P<0.01) when compared to a diseased negative control group. ***(P<0.001) when compared to a diseased negative control group.

DISCUSSION

Carrageenan-loaded paw edema and the tail-flick test are two experimental models that were used to evaluate the analgesic and anti-inflammatory effects of quercetin and its semi-synthetic derivatives. The pain effects of quercetin and its O-methylated derivatives, including 3,7-di-methyl-quercetin and 3,7,4′-tri-*O*-methyl-quercetin, were further evaluated using the tail immersion method. The tail immersion method also called heat stimulation creates supraspinal central regulated analgesia. The approach described above was facilitated through supraspinal routes and demonstrates a special selectivity that is intentionally tailored for analgesics with central action.

In the tail immersion method, it was observed that quercetin and its *O*-methylated derivatives, specifically 3,7-di-methyl-quercetin, and 3,7,4'-tri-*O*-methyl-quercetin, demonstrated a notable elevation in reaction time to thermal stimuli, indicating the presence of pain-inducing effects. Quercetin and its *O*-methylated derivatives demonstrate notable effects after 60 and 120 minutes when a dose administered orally of 0.1 g per kilogram as well as 0.2 g per kilogram. But when taken orally, no apparent effect is seen at the amount between 0.025 g and 0.05 g per kilogram of body weight. This methodology involves the consideration of an increase in reaction time as a means to assess the efficacy of central anti-nociceptive activities [19].

The use of the carrageenan-loaded rat paw edema study is an effective way to evaluate the efficacy of anti-inflammatory drugs, specifically assessing their anti-edematous properties. Carrageenan, a potent compound used in the induction of inflammatory and pro-inflammatory substances, such as leukotrienes, prostaglandins, histidine, Tumor Necrosis Factor Alpha, and bradykinin [20].

Inflammatory events are characterized by alterations in microvascular function, leading to heightened vascular permeability and the release of exudate, which includes plasma proteins. Additionally, there is an upregulation of endogenous chemical mediators, contributing to the amplification of the inflammatory response. Nonsteroidal anti-inflammatory medication can be useful in pharmaceutical agents for superficial nociception and inflammation. NSAIDs effectively mitigate hyperalgesia symptoms that are often associated with inflammation. This is achieved by the suppression of cyclooxygenase which is stopped to produce prostaglandins from arachidonic acid.

The most effective and widely employed approach for evaluating the anti-inflammatory properties of a substance is the induction of hind paw edema using carrageenan. The induction of hind paw edema by carrageenan is characterized by a bi-phasic response. The 1st stage begins with the release of serotonin, histamine, and kinins subsequent to the administration of a

phlogistic agent during the initial hours. The 2nd phase is characterized by the release of chemicals similar to prostaglandins within a time frame of 2-3 hours. The 2nd phase is responsive to both nonsteroidal anti-inflammatory and steroidal agents that have therapeutic utility. Prostaglandins are primarily accountable for the initiation and progression of acute inflammation. The subsequent phase was associated with the introduction of prostaglandins.

The study observed that Quercetin and its *O*-methylated derivatives exhibited a noteworthy ($P<0.001$) anti-inflammatory effect across all dosage levels at the acute carrageenan-loaded paw edema model. This test is recognized for its predictive value in assessing the efficacy of anti-inflammatory substances that function by inhibiting acute inflammation substances. In this experimental approach, the reference drug diclofenac sodium (orally delivered dosage of 0.01 g per kilogram) showed statistically significant effects in suppressing carrageenan-induced inflammation. These effects were observed at both the 2-hour and 3-hour time points following drug administration, as determined through analysis of three consecutive cases. Significant results were observed when diclofenac sodium (0.01 gram per kilogram) was administered to rats on three separate occasions, each involving a different drug.

Outcome of the research showed that quercetin and its *O*-methylated derivatives exhibited a notable decrease in inflammation, with this effect being observed in a dose-dependent manner during both the initial and subsequent phases. The results obtained from the experiment on carrageenan-loaded paw edema conducted in the previous study indicated that quercetin and its O-methylated derivatives, when used as test drugs, exhibit anti-inflammatory properties. The current findings of the experimental study suggest that quercetin and its *O*-methylated derivatives have the potential to exert peripheral effects.

CONCLUSIONS

The main goals of this work are to assess how quercetin act as NSAIDS and semi-synthetic derivatives when orally supplied to Wistar albino rats at dosages 0.1 and 0.2 g per kilogram. The current investigation has shown evidence that quercetin and its O-methylated derivatives, namely 3,7-di-methyl-quercetin and 3,7,4′-tri-*O*-methyl-quercetin acts as NSAIDS on way that was based on the dosage administered ($P<0.001$). Furthermore, it was noted that the oral administration of quercetin and its semi-synthetic derivatives at doses of 0.1 and 0.2 g per kilogram had similar efficacy to the conventional medicine diclofenac sodium, which was orally supplied at a dose of 0.01 g per kilogram. Optimized doses of 0.1 and 0.2 g per kilogram were determined to be both safe and substantial in terms of their biological efficacy. These findings provide a strong basis for conducting additional investigations in the pharmacology field.

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ETHCAL CONSIDERATION

The Institutional Ethical Committee of the NSHM Knowledge Campus, Kolkata in which the work was done has approved this work.

Conflict of interests

The authors declare no conflict of interests.

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