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ORAL PRESENTATION - FULL PAPER

COMPARISON OF THE EFFECTS OF QUERCETIN AND QUERCETIN-LOADED CYCLODEXTRIN FORMULATIONS ON ACUTE PAIN

Özge Özcan[1], Hazal Eken[2], Alper Öztürk[3], Nurcan Bektas[4], Rana Arslan[4]

[1]Graduate School of Health Sciences, Anadolu University, 26470, Eskisehir, Türkiye, ozge.ozcan@anadolu.edu.tr, https://orcid.org/0000-0001-6624-5439
[2]Department of Pharmacology, Faculty of Pharmacy, Afyonkarahisar University of Health Sciences, 03218, Afyonkarahisar, Turkey, E-mail: hazal.eken@afsu.edu.tr
[3]Department of Pharmaceutical Technology, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey
[4]Department of Pharmacology, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey

Abstract

Quercetin is in the class of flavanols that its chemical formula is 3.3', 4', 5,7-pentahydroxyflavone. It has been demonstrated to possess diverse pharmacological benefits, such as serving as an antioxidant, having antiviral properties, and exhibiting anti-cancer effects. Quercetin has also anti-inflammatory and antinociceptive/analgesic properties. However, the low solubility and bioavailability of quercetin adversely affect its effectiveness. Thus, in order to increase the bioavailability and solubility of quercetin, it is aimed to examine the change in the analgesic effect of quercetin by preparing a biologically compatible cyclodextrin formulation that can be targeted to the tissues in this study. Quercetin and quercetin-β-cyclodextrin (β-CD) complex were administered intraperitoneally at doses of 3, 5, 10, and 20 mg/kg in mice. Time-dependent analgesic effects of pure quercetin and quercetin-β-CD were compared in hot-plate and tail-immersion tests, acute pain models tested supraspinal and spinal organization of pain respectively. Quercetin at the doses of 5, 10 and 20 mg/kg was found to increase pain thresholds against to thermal stimuli in tail immersion and hot plate tests in 30-180 min. time interval. Only the effect of 20 mg/kg quercetin weakened at 180 min. The effect at 3 mg/kg was observed only in hot-plate test at 120 min. β-CD complex prepared with 3 mg/kg quercetin showed significant analgesic effect in 30-180 min time intervals in both two tests. Additionally, the analgesic effect of the β-CD complex prepared with 20 mg/kg quercetin was not weakened in both tests like pure 20 mg/kg quercetin at 180 minutes. Thereby, it can be concluded that application of quercetin in β-CD complex is provided to improve pharmacokinetic properties such as bioavailability of quercetin. Thus, the efficacy and duration of action of quercetin were enhanced. This study displays the analgesic potential of quercetin by spinal and supraspinal regulations and the advantages of application in β-CD formulation for pain relief.

Key Words: Cyclodextrin, β-Cyclodextrin, quercetin, pain, tail-immersion, hot-plate.

1. Introduction

Quercetin is a plant-derived flavonoid that belongs to the class of flavonols. Its chemical formula is 3.3', 4', 5,7-pentahydroxyflavone (Fischer et al., 1997). It is found abundantly in various fruits and vegetables, such as apples, berries, onions, and green leafy vegetables. Quercetin has gained considerable attention in recent years due to its potential health benefits. It has been shown to have a variety of pharmacological effects, including antioxidant, anti-inflammatory, antiviral, and anticancer properties (Mien and Mohammed, 2001; Grawaltney-Brant, 2006; Johari et al., 2012). One of the primary pharmacological effects of quercetin is its antioxidant activity. It can scavenge free radicals and prevent oxidative damage to cells and tissues. This may help chronic diseases such as cancer, heart disease, and neurodegenerative disorders. Quercetin has also anti-inflammatory activity (Boots et al., 2008; Lesjak et al., 2018). It can inhibit the production of inflammatory cytokines and enzymes, which may help to reduce inflammation in the body. It has been studied for its potential health benefits, including its analgesic effects (Lee vd., 2005; Filho vd., 2008; Chunmei vd., 2017). Quercetin may relieve pain is by blocking the transmission of pain signals in the nervous system (Ferraz et al., 2020; Ye et al., 2021).

Analgesics are drug or substances that relieve pain without causing loss of consciousness. Studies have shown that quercetin has analgesic effect in both animal and human models. In human studies, quercetin has been shown to reduce pain associated with conditions such as osteoarthritis and migraine headaches (Samadi et al., 2022). The analgesic effects of quercetin in experimental animals were first demonstrated by Rylski et al., (1979) and later by Picq et al., (1991). In different analgesia tests, quercetin has been shown to have an antinociceptive effect at doses of 10–60 mg/kg (i.p.) and 100–500 mg/kg, (p.o.) (Filho et al., 2008). It has been reported that cytokine inhibition and oxidative stress inhibition contribute to the analgesic effect of quercetin (Valério et al., 2009)

It is known that flavonoids are converted into conjugation forms such as glucuronides, sulfates and methylated derivatives after their absorption. It has been reported that these conjugates contribute to the therapeutic properties of flavonoids (Viskupicova et al., 2008). However, the bioavailability of quercetin can vary depending on several factors. One of them is quercetin's metabolism. Quercetin undergoes extensive metabolism in the liver and intestines before it can be absorbed into bloodstream. This can reduce its bioavailability and limit its effectiveness. Bioavailability refers to the amount of a substance that is absorbed by the body when consumed in food or supplements. To improve solubility and absorption, quercetin supplements can be often formulated with other compounds such as phospholipids or nanoparticles (Jaganath, et al., 2006; McClements vd., 2010).

Various drug delivery systems, such as lipid-based carriers, polymer nanoparticles, cyclodextrins, etc., are used to improve conditions such as solubility and bioavailability that limit the biological effect (Mainardes & Silva, 2004; Tiwari, et al., 2012). Cyclodextrins are substances in oligosaccharide structure that can change the physicochemical structures of the substances they form complexes with, through their lipophilic outer surfaces and hydrophilic interior spaces (Del Valle, 2004). Within the scope of this study; in order to increase the bioavailability and solubility of quercetin, it is aimed to examine the changes in the analgesic effect of quercetin by preparing a biologically compatible cyclodextrin formulation that can be targeted to the tissues.

2. Material and Methods

2.1. Materials

Quercetin and β-cyclodextrin (β-CD) were procured from Sigma-Aldrich (Steinheim, Germany), while all other chemicals utilized were of analytical grade. Deionized and filtered water was employed throughout all experiments (Milli-Q Academic, Millipore, Molsheim, France).

2.2. Experimental animals

Balb-c female mice weighing 25-35 grams were used in our experiments. Animals were obtained from Anadolu University Experimental Animals Research and Application Unit. Mice were housed in well-ventilated rooms with day/night lighting for 12 hours before the experiments. The animals were allowed to freely feed and drink water. Analgesia tests were conducted during the hours of 11:00 AM to 5:00 PM. Our experimental procedure was approved by the local ethics committe of Anadolu University, Eskisehir, Turkey (11.12.2019 - 2019-57).

2.3. Drug administration

Quercetin was dissolved in 10% DMSO and injected into (ip) mice 30 minutes before the experiment at doses of 3, 5, 10 and 20 mg/kg in a volume of 0.1 ml. The control group was given 10% DMSO in a volume of 0.1 ml.

2.4. Formulations

In this study, the formulation production technique of solvent evaporation was chosen. (Jantarat et al., 2014; Öztürk et al., 2023). According to Higuchi and Connors' phase solubility experiments, a quercetin-containing β-CD complex was made using the solvent evaporation method in an ethanol:water (1:1, v/v) solvent system. Firstly, the mixtures were strongly shaken at 300 shake / min−1 during the equilibrium period as being protected from direct sunlight. After the shaking period, the mixtures were dried at a controlled temperature and pressure with Büchi R-205 (Switzerland) rotary evaporator. In this case, the temperature was set to 50°C ± 1°C and the pressure was set to 100 mbar .

Table 1. Lists the ingredients in the made formulation.

Formulation code	Quercetin	β-CD	Ethanol	Water
Q- β-CD	10 mg	37.58 mg	32 mL	32 mL

2.4.1. Characterization of formulation

HPLC was used to determine the concentration of quercetin in the complex. To carry out the analysis, an accurately weighed formulation of 1 mg is dissolved in 1 mL of a mobile phase and then HPLC analyses were performed. Encapsulation efficiency was expressed in terms of associated drug percentage (Öztürk et al., 2023, Perret et al., 2013).
At 25 °C ± 2 °C in distilled water, zeta potential studies were carried out using the Zetasizer Nano ZS (Malvern, UK) (Tilki et al.,2023; Baghirova, et al., 2023).

2.4. Experimental methods

2.4.1. Hot plate test

The hot plate test is used to assess the response of experimental animals to thermal painful stimuli (Eddy and Leimbach, 1953; Uzbay, 2004). This test is related to higher brain functions and supraspinal organized response (Dzoyem et al., 2017). Animals were handled gently and placed on the heated surface of the hot plate apparatus (No. 7280, Ugo Basile Instruments, Comerio, Italy) set to 56 degrees. The latency to hind paw licking, hind paw flicking or jumping, was recorded, and at this point the animal was immediately removed from the hot plate. After baseline measurement of animals' pain

response, post-drug latency was measured after the administration of the test compounds in experimental groups, and vehicle in the control group. Time intervals of the measurements after administration of the compounds were chosen as 30, 60, 120, and 180 min. The cut-off time was determined at 20 seconds to prevent hind paw damage (Eddy and Leimback, 1953; Langfor and Mogil, 2008).

2.4.2. Tail-immersion test

The tail-immersion test is a commonly used experimental procedure for assessing thermal nociception in experimental animals. In this test, approximately 3 cm from the distal end of the animals' tails were immersed in a water bath heated to 52.6°C to determine the nociceptive response. In the tail-immersion test, the time it takes for the animal to withdraw its tail from the thermal stimulus is measured as the reaction time (Aydın et al., 2002; Gawel et al., 2018). After baseline measurement of animals' pain response, post-drug latency was measured after the administration of the test compounds in experimental groups, and vehicle in the control group. Time intervals of the measurements after administration of the compounds were chosen as 30, 60, 120, and 180 min.. Time intervals of the measurements after administration of the compounds were chosen as 30, 60, 120, and 180 min The cut-off time of 15 s was set to prevent harm to tail tissue (Ramabadran et al., 1989; Aydın et al., 2002).

2.5. Statistical analysis

Hot plate and tail immersion test results were analysed by use of Two-way ANOVA test in the GraphPad Prism version 5 statistical programe. Values are given mean±SEM and $p < 0.05$ was considered statistically significant.
The hot-plate and tail-immersion tests produced results that were represented as a percentage of the maximum possible effect (MPE %). The MPE % was determined by calculating the response latencies to thermal stimuli and using that value as the basis for the percentage calculation:

$$MPE\% = [(Postdrug\ latency) - (Predrug\ latency)]/\ [(Cutoff\ time) - (Predrug\ latency)] \times 100$$

3. Results and Discussion

3.1. Formulation

The sum of the masses of the dried complex and the amount of quercetin and β-CD used was calculated as the complexation performance. The Q-β-CD coded formulation was produced with a yield of 65.5% ± 4.9%. The aqueous solubility and encapsulation efficiency of quercetin were obtained as 27.5 $\mu g.mL^{-1}$ and 84.0 ± 4.2%, respectively. The aqueous solubility of quercetin is 1.5 $\mu g.mL^{-1}$, and it has been determined that it increases considerably when complexed with β-CD (Marques et al., 2019). Because greater loading results in a lower quantity of formulation for a given dose of treatment, the drug encapsulation efficiency of the formulation is a crucial factor in formulations. The result of 84.0 ± 4.2% obtained in this study is quite ideal (Ekinci et al., 2022). The zeta potential of the Q-β-CD -coded formulation was obtained as −19.5 ± 0.2 mV. In colloidal systems, the particles resist one another if the zeta potential is strongly positive or negatively charged. If a colloidal dispersion has a zeta potential of ± 30 mV when dispersed in a liquid, it is assumed to be a stable formulation. Zeta potential values between 5.0 and -15.0 mV are considered in the limited flocculation zone, while zeta values between -5.0 and -3.0 mV are considered the maximum flocculation zone. When the study's findings were investigated, it was discovered that the zeta potential values were higher than the flocculation limit. This demonstrates the formulation's stability (Öztürk et al., 2023). When all these results are evaluated, especially the increase in water solubility of quercetin due to the complex will increase the bioavailability of quercetin (Carnerio et al.,2019).

3.2. In vivo Experiments

The objective of this study was to evaluate and compare the analgesic properties of pure quercetin and a Q-β-CD formulation. It was hypothesized that the use of cyclodextrin could enhance the pain-relieving effect of quercetin by improving its solubility and increasing its bioavailability. Antinociceptive effect of pure quercetin has been investigated dose dependently (3, 5, 10, 20 mg/kg) on hot plate and tail immersion tests. Q-β-CD formulation was studied at the same dose range on the hot plate and tail immersion tests.

In the hot plate test, which is a behavioral model of nociception, paw licking, mustache movements and jumping are observed, which are controlled by supraspinal mechanisms. In this test, a significant antinociceptive effect was observed that the quercetin at doses of 5, 10, 20 mg/kg compared to the control group at all time intervals (30-180 min.) ($^{c}p< 0.001$, Figure 1). No analgesic effect was observed at a dose of 3 mg/kg of pure quercetin. However, it was determined that the effect of 20 mg/kg dose, where the highest effect was seen, is reduced by half in the 180th minute. Despite the decrease in dose to 20 mg/kg, the antinociceptive effect was still present when compared to the control group.

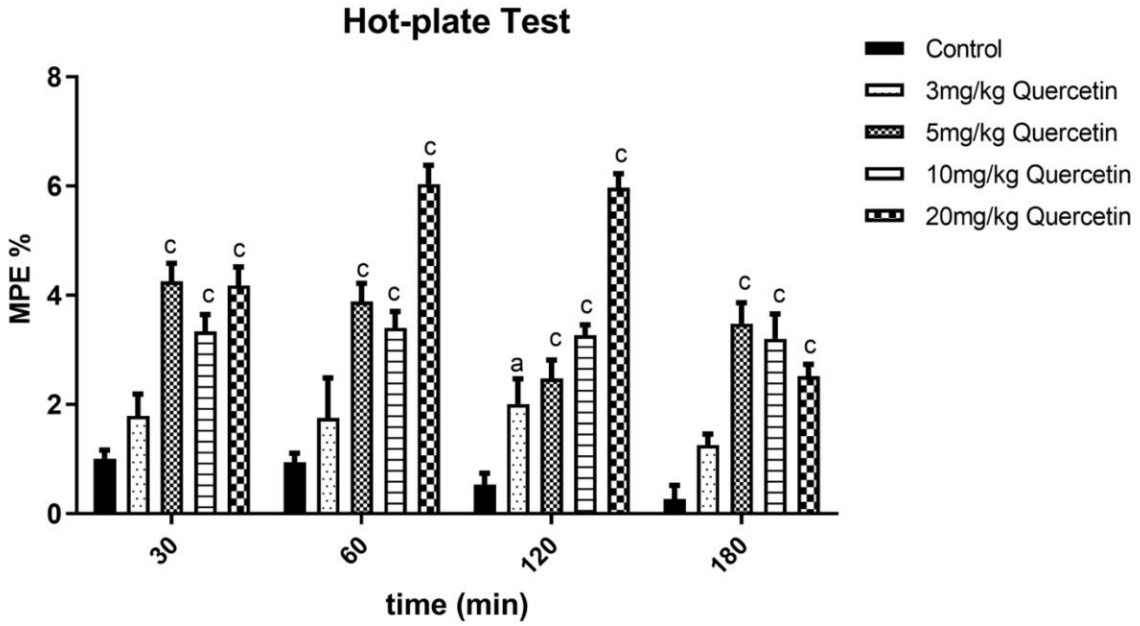

Figure 1. Antinociceptive effect of quercetin in hot-plate test. Compared with the control group: $^{a}p< 0.05$., $^{c}p< 0.001$. Values are expressed as the mean ± S.E.M. (n = 8)

At the same doses of quercetin was found to increase pain thresholds against to thermal stimuli in tail immersion test in 30-180 min time interval ($^{c}p< 0.001$, Figure 2). Similar to the hot plate test results, the antinociceptive effect obtained at all doses decreased at the 180th minute.

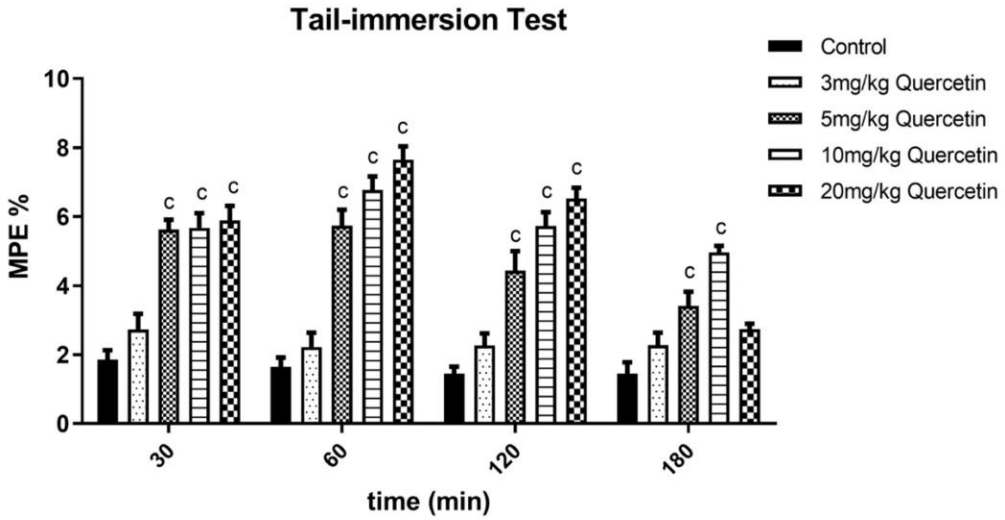

Figure 2. Antinociceptive effect of quercetin in tail-immersion test. Compared with the control group: $^{c}p< 0.001$. Values are expressed as the mean ± S.E.M. (n = 8)

When pure quercetin and the Q-β-CD coded formulation was compared, it was determined that the 3 mg/kg Q-β-CD coded formulation increased the analgesic efficacy between 30-180 minutes in the hot plate test (Figure 3). The analgesic activity of the 3 mg/kg Q-β-CD formulation has significant difference compared to the pure 3 mg/kg quercetin in the hot plate test ($^{*}p< 0.05$ and $^{***}p< 0.001$, Figure 3). Similarly, it was observed that the analgesic effect of 20 mg/kg Q-β-CD formulation continued at the same time interval and also significantly higher compared to pure quercetin ($^{\&\&}p< 0.01$ and $^{\&\&\&}p< 0.001$, Figure 3). Comparing analgesic activities of 5 and 10 mg/kg quercetin with their Q-β-CD formulation showed no significant difference at all time intervals. Thus, application of quercetin in formulation provides an advantage to supraspinal controlled analgesic effect of quercetin.

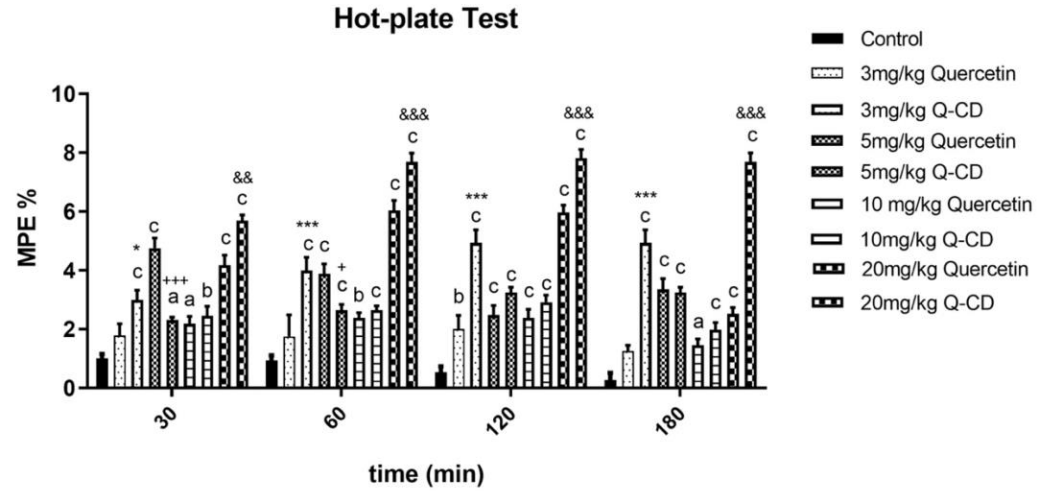

Figure 3. Comparison of pure quercetin and cyclodextrin-quercetin formulation application in hot plate test. Significant difference compared to the control group: $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$. Comperison with pure quercetin and Q-CD formulation-3mg/kg : $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; Comperison with pure quercetin and Q-CD formulation 20mg/kg: $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$. Values are expressed as the mean ± S.E.M. (n = 8) (Q-CD: Quercetin-β Cyclodextrin formulation)

In the tail immersion test, when the antinociceptive effect of pure quercetin and Q- β-CD formulation is compared with each other at 3 mg/kg doses, it was seen that the formulated groups have more significant analgesic effects between 60-180 minutes ($^{*}p< 0.05$, $^{**}p< 0.01$; Figure 4). At the dose of 20 mg/kg, the formulation provided a significant increase in analgesic effect, especially when compared to the reduced pure quercetin effect at the 180th minute ($^{\&\&\&}p< 0.001$, Figure 4). The cyclodextrin formulation did not provide a significant difference in the analgesic effect of 5 and 10 mg/kg quercetin. In both analgesia tests, only the analgesic effect of 5 mg/kg Q-β-CD formulation showed significant reduction (Figure 3-4). However, it can be said that application of quercetin in complex folmulation provides an advantage to spinally controlled analgesic effect of quercetin.

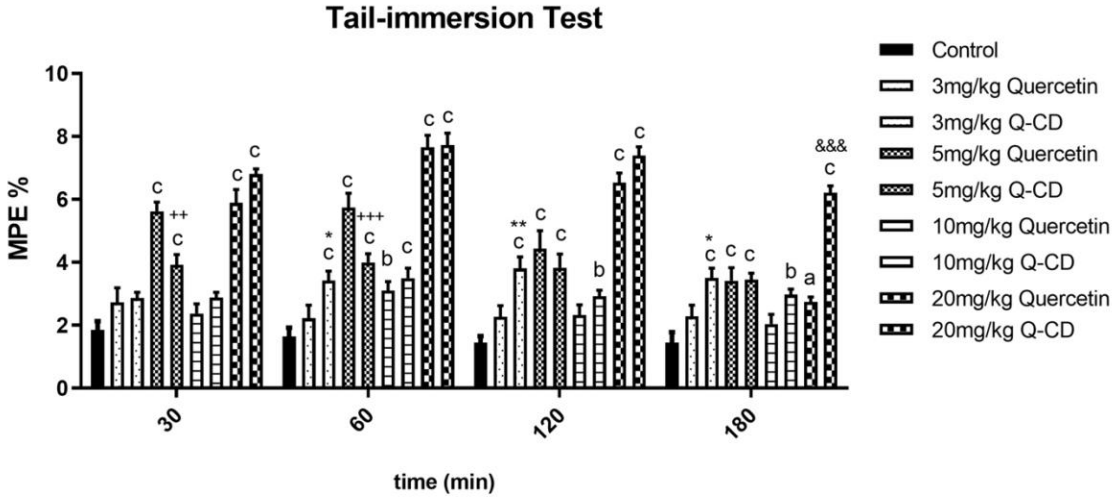

Figure 4. Comparison of pure quercetin and cyclodextrin-quercetin formulation application in tail immersion test. Significant difference compared to the control group: $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$. Comparison with pure quercetin and Q-CD formulation-3mg/kg : $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; Comparison with pure quercetin and Q-CD formulation 20mg/kg: $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$. Values are expressed as the mean ± S.E.M. (n = 8) (Q-CD: Quercetin-β Cyclodextrin formulation)

As stated earlier, quercetin is a bioactive substance with many important pharmacological effects. However, its pharmacokinetic properties, which cause low bioavailability, set the limits for its use as a drug (Cai, et al., 2013). Various strategies could be employed to enhance the bioavailability of quercetin, including the utilization of advanced drug delivery systems. These systems have shown promising results in improving the solubility and bioavailability of quercetin (Cai, et al., 2013).

Drug delivery systems are designed to deliver the necessary amount of medication to the target area effectively and accurately over a required period. Additionally, suitable carrier materials are used to design advanced dosage forms and improve the undesirable properties of drug molecules (Loftsson et al., 2005; Adepu and Ramakrishna, 2021). Cyclodextrins are an oligosaccharide carrier system widely used in drug development to solve problems such as absorption, stability and bioavailability. Cyclodextrins facilitate the formulation of drugs by providing a more stable and homogeneous distribution of drugs. In this way, they enable drugs to affect the targeted tissues more easily. Cyclodextrins are water-soluble drug carriers with a hydrophobic core and a hydrophilic outer surface, which make them suitable for delivering hydrophobic injectable drugs (Uekama, et al., 1998). Additionally, it has been demonstrated that this carrier system provides advantages to biological effects of various flavonoids by developing pharmacokinethic properties (Rezende et al., 2009;

Tommasini, et al., 2004). So, cyclodextrin carrier system was choosen for improve bioavalibility of quercetin in this study and successful results were obtained.

In our results, while 3 mg/kg pure quercetin has no analgesic effect, Q-β-CD formulation increase the quercetin analgesic effect at the lowest dose (3 mg/kg) and the formulation allowed this effect to continue until the 180th minute. At the same time, the decreasing effect of 20 mg/kg quercetin at 180 minutes was enhanced by the cyclodextrin complex and mediated the increase of this effect at all time intervals. Briefly, cyclodextrin provided that demonsrate analgesic effect of quercetin at low doses, with an early onset and prolonged duration of action.

When all findings are considered, it was determined that the quercetin β-CD complex increased the analgesic effect of quercetin and prolonged its duration of action. The cyclodextrin carrier system is thought to facilitate a controlled release of quercetin, enabling it to more efficiently reach the target tissues and thereby enhance its efficacy.

4. Conclusion

Recently, quercetin, a notable flavonoid, was loaded into the cyclodextrin carrier system to increase its efficiency and bioavailability. Results of the present study indicated that the cyclodextrin complex enhanced the analgesic effect of quercetin and prolonged its duration of action.

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Conflict of Interest
The authors of this study do not have any conflicting interests.

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